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March 2000

NATIONAL RESEARCH INITIATIVE
COMPETITIVE GRANTS PROGRAM

ABSTRACTS OF FUNDED RESEARCH
Fiscal Year 1999

TABLE OF CONTENTS

INTRODUCTION iii

ABSTRACTS OF FUNDED RESEARCH i
NATURAL RESOURCES AND THE ENVIRONMENT 3
- ECOSYSTEM SCIENCE 5
- PLANT RESPONSES TO THE ENVIRONMENT 12
- SOILS AND SOIL BIOLOGY 21
- WATER RESOURCES ASSESSMENT AND PROTECTION 28

NUTRITION, FOOD SAFETY, AND HEALTH 37
- IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH 38
- ENSURING FOOD SAFETY 47
- EPIDEMIOLOGICAL APPROACHES FOR FOOD SAFETY 56

ANIMALS 60
- ANIMAL REPRODUCTIVE EFFICIENCY 61
- ANIMAL GROWTH, DEVELOPMENT, AND NUTRIENT UTILIZATION 70
- ANIMAL GENOME AND GENETIC MECHANISMS 76
- ANIMAL HEALTH AND WELL-BEING 83

PEST BIOLOGY AND MANAGEMENT 102
- PLANT PATHOLOGY 103
- ENTOMOLOGY AND NEMATOLOGY 114
- WEED BIOLOGY AND MANAGEMENT 129
- BIOLOGICALLY BASED PEST MANAGEMENT 133

PLANTS 139
- PLANT GENOME 140
- PLANT GENETIC MECHANISMS 146
- PLANT GROWTH AND DEVELOPMENT 156
- PHOTOSYNTHESIS AND RESPIRATION 168
- NITROGEN FIXATION/NITROGEN METABOLISM 174

MARKETS, TRADE, AND RURAL DEVELOPMENT 179
- MARKETS AND TRADE 180
- RURAL DEVELOPMENT 189

ENHANCING VALUE AND USE OF AGRICULTURAL AND
- FOREST PRODUCTS 194
- FOOD CHARACTERIZATION/PROCESS/PRODUCT RESEARCH 195
- NON-FOOD CHARACTERIZATION/PROCESS/PRODUCT RESEARCH 205
- IMPROVED UTILIZATION OF WOOD AND WOOD FIBER 211

AGRICULTURAL SYSTEMS 218
STRENGTHENING AWARDS 225
POSTDOCTORAL FELLOWSHIPS 275
NEW INVESTIGATOR AWARDS 283

CONFERENCE GRANTS 297
U.S. RICE GENOME SEQUENCING PROJECT (USDA/NSF/DOE) 306

APPENDICES 311
APPENDIX A.
Statistics for the 1998 Fiscal Year 313
APPENDIX B.
Scientists Who Served on NRI Peer Review Panels for Fiscal Year 1998 317

INDEX OF INVESTIGATORS 325

INTRODUCTION

In the foreword to last year's Abstract book, I wrote about the need for further investment in the national agricultural research enterprise despite the fact that food prices relative to personal income were at an all time low and that supermarkets were brimming with an abundance of varied, affordable food. That need has not changed. Discovery research and the conversion of the acquired knowledge into new technologies are needed if the U.S. agricultural enterprise is to remain competitive in world and domestic markets. Only science-based methods will allow farmers and ranchers to reduce their dependency on pesticides and antibiotics, combat new and emerging diseases and pests, protect the water supply, soils and fragile ecosystems, and bring new, agriculturally-based industries to rural communities. Not to invest in agricultural sciences would be as shortsighted as not providing an increased budget to the NIH, simply because the state of the nation's health is at an all time high and life expectancy continues to rise. Indeed, an argument could be made that the availability of high quality, safe and affordable food has been as much of a contributing factor to good health as public health measures and antibiotics. Success should never lead to complacency. Investment is as much needed now as it was over a century ago when the land grant system of research universities was established.

1999 was also the year that the acronym GMO made its entrance into the vernacular, raising public concerns about where agricultural science is heading and whether research that underpins such technological breakthroughs acts in the common good. I would argue that the National Research Initiative (NRI) Competitive Grants Program (and its predecessor programs within the USDA) should be proud of the discoveries they have sponsored in the general areas of molecular genetics. Such research has undoubtedly contributed to the ease with which organisms important to agriculture can be modified by shuffling around genes, sometimes across breeding barriers, but it has also identified some of the risks that can be incurred and how such risks can be minimized. It is my hope that NRI programs will continue to involve themselves in discoveries that can help farmers around the world produce the calories and nutrition needed to support 10 billion or more people by the mid 21st century. Now is certainly not the time to turn away from a technology that has potential for such good.

Agricultural research sponsored by the USDA is organized to provide five broad outcomes:

- An agricultural system that is highly competitive in the global economy
- A safe and secure food and fiber system
- A healthy well-nourished population
- Greater harmony between agriculture and the environment
- Enhanced opportunities for farmers, ranchers, and rural people and communities.

The NRI Competitive Grants Program, which is administered through the Cooperative State Research Education and Extension Service (CSREES), is the major competitive

grants program within the USDA research and development portfolio, although representing only about 7% of the Department's total research & development budget. The NRI is dedicated to the USDA outcomes listed above. To achieve its goals, the NRI attempts to attract research proposals from the highest qualified U.S. scientists, whether or not they are from a traditional college of agriculture. In 1999, for example, approximately 18% of the submitted proposals came from private and public colleges and universities that are not Land-Grant institutions, 4% from private organizations and 5% from Federal agencies, including the Agricultural Research Service (ARS), which is the intramural research arm of the USDA. These proposals are subjected to competitive peer-review by assembled panels of experts, as well as by peers from around the world. Last year the NRI drew upon the services of several thousand such experts who judged the proposals on the basis of their scientific merit and originality, as well as on their relevance to long-range improvements and sustainability of U.S. agriculture. Great care is taken to avoid real and perceived conflicts of interest in the peer-review process. Moreover, the NRI always bases its awards on the rankings provided by its panels of reviewers. Post-panel adjustments are not made. The quality of the science is judged paramount.

The *NRI Program Description* is distributed widely within the scientific community and among other interested groups, usually in late summer. The Fiscal Year (FY) 1999 *Program Description*, published in the *Federal Register* on August 28, 1998, identified 28 research programs within the following eight major research areas:

- Natural Resources and the Environment
- Nutrition, Food Safety, and Health
- Animals
- Pest Biology and Management
- Plants
- Markets, Trade, and Rural Development
- Enhancing Value and Use of Agricultural and Forest Products
- Agricultural Systems Research

After years of virtually zero growth, the NRI received a substantial (~ $20 million) increase in its budget for the 1999 fiscal year. This addition allowed it to increase the size and duration of many awards over what had been possible previously and also to fund some new, important initiatives in genome research and food safety.

The NRI supports both "fundamental" and "mission-linked" research. Fundamental research tests new ideas and seeks basic scientific information on agriculturally important issues. Mission-linked research provides scientific understanding needed specifically to solve important current problems relevant to agriculture, food, forestry and the environment. Approximately half of the FY 1999 support was made to fundamental projects; the remaining half was judged mission-linked. The NRI encourages the kinds of multi-disciplinary research that are needed to solve complex problems and to initiate

research in new areas of science and engineering that are relevant to agriculture, food, forestry and the environment. Multi-disciplinary research accounted for 43.4% of awards in FY 1999.

The majority of awards in FY 1999 were new standard research projects (excludes Research Career Enhancement Awards, Equipment Grants, Seed Grants, conferences, continuing increments, and supplements). The average grant in this category was $165,224, with an average duration of 2.2 years--for FY 1998, these figures were $146,666 for 2.3 years. The increases in grant size and duration were due in part to the improved NRI budget for FY 1999.

Two needed new programs were initiated in 1999. The first was a fledgling animal genome initiative designed to provide resources and tools, such as expressed sequence tags (ESTs) for agriculturally important animal species, improved libraries, and better genetic maps, to the scientific community. The second was for epidemiological studies on food safety, which was established in response to the doubling of the NRI budget for Food Safety. Both programs were planned to attract teams of investigators, sometimes from several institutions. As a result the grants awarded were much larger than the standard research projects traditionally supported by the NRI. The nine food safety grants, for example, averaged $590,230, with the largest being $885,294.

The NRI partially supported 25 conferences in FY 1999 with grants totaling $189,101. These conferences brought scientists together to identify research needs and to update one another on the latest research information and advances in areas important to U.S. agriculture. Also in FY 1999, the NRI provided funds totaling $18,933,478 for Agricultural Research Enhancement Awards (AREA), which included Postdoctoral Fellowships, New Investigator Awards, and Strengthening Awards. This innovative program assists institutions in developing or strengthening the competitiveness of their research programs and in attracting new scientists to careers of national needs in the food, agricultural, and environmental sciences. Strengthening Awards are available to small and mid-sized academic institutions or to institutions in the USDA-EPSCoR states. Four types of Strengthening Awards are available: 1) Research Career Enhancement Awards that support sabbatical leaves; 2) Equipment Grants for the purchase of equipment; 3) Seed Grants to enable investigators to collect preliminary data in preparation for applying for a standard research grant; and 4) Strengthening Standard Research Project Awards that fund research grants at eligible institutions. The NRI reserves at least 10% of its available funds for AREA awards.

Finally, the NRI participated in interagency programs in FY 1999: the *Arabidopsis* Genome Sequencing Project (National Science Foundation (NSF), Department of Energy (DOE), and USDA), and the Interagency Metabolic Engineering Program (DOE, NSF, Department of Commerce (DOC), Department of Defense (DOD), Environmental Protection Agency (EPA), and National Aeronautics and Space Administration (NASA)).

The funding equivalent of one or two grants is provided to each of these programs. The NRI (with NSF and DOE) also spearheaded the U.S. portion of the International Rice Genome Project in FY 1999. Two grants totaling $12.3 million were made for three years to provide the full sequence of rice chromosome 10.

This report provides brief descriptions, provided by the investigators, of the 690 new research grants funded by the NRI in Fiscal Year 1999. I am proud of the quality of these proposals and of the peer review process that ranked them. I am especially grateful to the dedicated staff-scientific, administrative and clerical-who make the NRI work so well in the service of U.S. agriculture.

R. Michael Roberts
Chief Scientist
National Research Initiative Competitive Grants Program
Cooperative State Research, Education, and Extension Service
United States Department of Agriculture

ABSTRACTS OF FUNDED RESEARCH

NATURAL RESOURCES AND THE ENVIRONMENT

Research in the area of natural resources and the environment addresses contemporary issues important for agriculture and for society. Knowledge in diverse scientific disciplines is needed in order to understand the influence of environmental fluctuations, to foster sustainability and economic viability, and to enhance stewardship of natural resources and agriculturally important ecosystems. Program Areas in this Division include: Plant Responses to the Environment; Ecosystem Science; Soils and Soil Biology; and Water Resources Assessment and Protection. The Natural Resources and Environment Division also provides funding for the Improved Utilization of Wood and Wood Fiber Program; abstracts for this program can be found in the Enhancing Value and Use of Agricultural and Forest Products Division section.

ECOSYSTEM SCIENCE

Panel Manager - Dr. Linda Wallace, University of Oklahoma
Program Director - Dr. Michael O'Neill

The Ecosystem Science Program is designed to increase our understanding of interactions between abiotic and biotic components of ecosystems. This basic information is essential to assess environmental conditions and the sustainability of agriculture within an ecosystem context. Research on the structure, function and sustainability of forest, rangeland, crop, or aquatic (including riparian, wetland and estuarine areas, but not oceanic) ecosystems is supported. Results provide better information and tools for agricultural planning, evaluating the effects of agricultural practices on environmental quality and sustainability, and characterizing the functional integrity of natural ecosystems.

9900941 The Role of Organic Nitrogen in the Nitrogen Economy of Taiga Trees
Kielland, K.; Boone, R.; Ruess, R.
University of Alaska, Fairbanks; Institute of Arctic Biology; Fairbanks, AK 99775-0180
Strengthening Award; Grant 99-35101-7859; $467,500; 3 Years

Contrary to conventional wisdom, the conversion of organic nitrogen to inorganic forms is not a prerequisite for plant uptake. Plants from a variety of ecosystems, including several important agricultural species, have been shown to have the capacity to absorb organic nitrogen directly. In the context of low-input sustainable agriculture and forestry, these findings are extremely important. Here we propose to investigate if organic nitrogen plays a significant role in the nitrogen economy of taiga ecosystems. The research is based on four important observations: First, data for ecosystem nitrogen budgets in taiga forests show that the annual plant nitrogen requirements significantly exceed the annual inorganic nitrogen flux. Second, some taiga species have been shown to absorb organic nitrogen under field conditions. Third, taiga soils have large concentrations of soluble organic nitrogen. Fourth, inter-specific variation in the stable isotope chemistry of taiga trees suggests that plants may derive nitrogen from several different sources, including organic forms. The plan calls for a combination of intensive and extensive studies across primary (floodplain) and secondary (upland) successional sequences in the Alaskan taiga. The research will include: (1) uptake of organic nitrogen in the field, (2) determination of species-specific variation in $\delta^{15}N$ signatures, (3) turnover studies of organic and inorganic soil nitrogen fractions, and (4) ecosystem nitrogen budget determinations. The proposed research should advance our knowledge regarding the fundamental mechanisms of nitrogen cycling in taiga forests, and has profound ramifications for both global change research on natural ecosystems and applied agricultural practices.

9900921 Controls on CH_4 Emissions and Carbon Sequestration in a Paddy Field: A Multi-disciplinary Approach Combining Stable Isotopes, Radiocarbon, and Eddy Co-variance Measurements
Tyler, S.C.; Trumbore, S.E.; Goulden, M.L.
University of California, Irvine; Earth System Science Department; Irvine, CA 92697-3100
Grant 99-3501-7809; $404,000; 3 Years

We are conducting a multi-faceted measurement program in a California rice paddy aimed at answering questions regarding greenhouse gas emissions of CH_4 and CO_2. These questions arise as a result of rice agriculture growing practices and their relationship to CH_4 emissions and carbon storage, i.e., the sequestering of atmospheric CO_2 in wetlands as accumulated soil carbon. We will study how rice straw fertilizer affects paddy field CH_4 emissions by studying control (inorganic fertilizer only) and rice-straw-amended paddy fields. In paddies representing either treatment we will measure CH_4 emissions over the growing season as well as CH_4 concentration and stable C isotope ratios in sediment and plant stem gases to determine how processes of CH_4 production and oxidation are controlling CH_4 emissions. We will also address recent theoretical and/or calculational studies which indicate flooded agricultural lands in the northern hemisphere, including rice paddies, may be a large, as yet unaccounted for, sink for atmospheric CO_2 (i.e., paddy fields may sequester C). We will do this using two experimental methods. First by determining net carbon balance using year round micrometeorological measurements of CO_2 and CH_4 flux, and second, by determining carbon accumulation using measurements of natural abundance radiocarbon (^{14}C) in soil organic matter. The results of our study will be important for both U. S. agricultural concerns and for global concerns regarding CO_2 and CH_4 greenhouse gas emissions. Scientific investigations aimed at settling questions such as those posed here are necessary so that the United States can properly represent itself at international meetings designed to settle issues on global climate change.

9901077 Structure of Mycorrhizal Fungal Communities: A Test of Models
Parker, V.T.
San Francisco State University; Department of Biology; San Francisco, CA 94132
Grant 99-35101-7779; $237,000; 3 Years

I propose to investigate the processes determining the organization of fungi mutualistic with the roots of shrubs and trees. These fungi form mycorrhizal associations with plants, and enhance plant survival and growth. Much of my mycorrhizal research has investigated a positive feedback model that includes three components: the economically important Douglas-fir, *Pseudotsuga menziesii*, a second plant host, *Arctostaphylos*, and their mycorrhizal fungal mutualists. The *Arctostaphylos* mycorrhizal fungal community facilitates the establishment of Douglas-fir and strongly influences vegetation dynamics. My past work has discovered a high diversity fungal community associated with *Arctostaphylos*, but one dominated by a small number of species. I developed two different models of the community structure, differing in critical assumptions. The two models can be differentiated by a disturbance to the system. I propose to use fire as that disturbance, as it can modify both plant host and soil resource conditions, both important components to the models. The proposed research seeks to understand what processes are principally controlling the structure of this mycorrhizal community. We know some processes influence mycorrhizal fungal composition and abundance, including litter and soil development, ability to decompose different types of litter, moisture conditions, soil chemistry, biotic interactions, and disturbance. Results of this study apply directly to the management and conservation of Douglas-fir forests and associated chaparral. The project is significant in its general applicability to other systems and makes an important first step in understanding which processes principally influence patterns of mycorrhizal fungal abundance, frequency, and richness.

9900966 The Role of Cattle Grazing in Conserving Grassland Biodiversity
Holl, K.D.
University of California, Santa Cruz; Environmental Studies Department; Santa Cruz, CA
Strengthening Award; Grant 99-35101-8234; $125,000; 3 Years

This research investigates the potential for using cattle to restore and manage grassland ecosystems and their associated annual wildflowers. California coastal prairie is a threatened ecosystem that evolved with disturbances such as ungulate grazing and fire; in recent history, cattle have been a dominant component of disturbance regimes in coastal prairie. Understanding the effect of cattle grazing on annual wildflowers is of particular importance because these plants comprise much of the species diversity in coastal prairie, and many are at risk of extinction. This research consists of two complementary components: a large-scale survey and manipulative experiments. First, adjacent grazed and ungrazed sites will be surveyed throughout north/central, coastal California to compare the effects of cattle grazing on annual wildflower populations over a regional scale. Second, various treatments within cattle exclosures will test the effects of different grazing regimes, thatch removal, and soil disturbance. The effects of these treatments will be studied with respect to both the extant annual wildflowers and to the seeds, seedlings, and adults of two focal species, the endangered Santa Cruz tarplant (*Holocarpha macradenia*) and bearded clover (*Trifolium barbigerum* var. *barbigerum*). This research will expand our understanding of the effects of cattle grazing on annual wildflower populations. Coastal prairie management using livestock has the unusual and promising potential for meeting short- and long-term needs for agricultural production and maintaining biological diversity.

9900947 Biological Invasions and Changes in Ecosystem Structure and Functioning
Vitousek, P.M.
Stanford University; Department of Biological Sciences; Stanford, CA 94305-5020
Grant 99-35101-7733; $419,500; 3 Years

This project will evaluate how forests can be altered by the introduction of species from other parts of the world, and evaluate the implications of such changes for forest productivity. It makes use of a range of Hawaiian forests that differ substantially in soil fertility and productivity, as a consequence of differences in climate and soil age. All of these forests are dominated by the native Hawaiian tree *Metrosideros polymorpha*, which occupies an extraordinarily broad array of environments. Like other trees, *Metrosideros* both shapes and is shaped by the environments it occupies. It cannot be equally well adapted to all of the wide range of sites it dominates; how can the introduction of other species change both forest growth and soil fertility? This research evaluates young soils where forest production is limited by nitrogen (and a biological N fixer has invaded), fertile intermediate-aged soils (where a Mesoamerican tree adapted to fertile soils is invading), and old phosphorus-limited soils (where deep-rooted Australian trees are invading). Determining how these species change forest ecosystems will contribute to understanding how the presence of a diversity of species shapes forest ecosystems; it also will be useful for understanding how production of managed forests (where humanity controls species composition) can be sustained across a range of environments.

9900968 Influence of Marine Nutrients from Salmon on Stream Ecosystems
Lamberti, G.A.
University of Notre Dame; Department of Biological Sciences; Notre Dame, IN 46556-0369
Grant 99-35101-8592; $410,500; 3 Years

Every year, millions of salmon migrate into streams and rivers of the Pacific Northwest, where they die soon after spawning. For example, densities of dead salmon can exceed 1 fish per square meter in Alaskan streams. These migrations deposit carbon, nitrogen, phosphorus, and other nutrients from the ocean into spawning streams in the form of the salmons' decomposing bodies. Many organisms, ranging from fly larvae to bears, consume this resource. Although we know that salmon nutrients can be traced into stream food webs (algae, invertebrates, and young fish), and even into riparian vegetation, we do not know if salmon provide an essential nutrient 'pulse' for the stream ecosystem or if such nutrients can be replaced by other sources. This research will determine the influence of these marine-derived nutrients from spawning salmon on the productivity and sustainability of stream food webs in forested watersheds. Streams in the Tongass National Forest in southeast Alaska will be studied, because salmon runs in many such streams are still relatively intact whereas salmon runs are in decline in much of the remainder of the Pacific Northwest. We also will determine if forest management (old-growth vs. harvested) affects the role of salmon nutrients in stream productivity. Our working hypotheses are that (1) salmon nutrients provide an essential marine subsidy for stream food webs in forested streams, and (2) positive feedbacks ensure that streams with healthy salmon runs are more productive than streams with degraded runs, thereby ultimately producing more fish biomass.

9900937 Nitrogen Fertilization Effects on Soil Organic Matter Dynamics in a Corn-Belt Agroecosystem
Russell, A.E.
Iowa State University; Department of Botany; Ames, IA 50011-1020
Postdoctoral Fellowship; Grant 99-35101-8591; $90,000; 2 Years

Human activities have altered the global carbon cycle in profound ways. Nitrogen fertilization is one agricultural practice that can influence soil carbon stocks and atmospheric inputs by altering the quantity and/or quality of carbon and nitrogen inputs from crop roots and aboveground residues. Nitrogen fertilization can affect losses of carbon and nitrogen from soil by influencing the breakdown of soil organic matter (SOM) to release CO_2. Relationships between fertilization rate and grain production are well-studied, but surprisingly, relationships between excessive fertilization, root production and CO_2 losses are poorly understood. The objectives of this research are to quantify effects of increasing rate of nitrogen fertilization on carbon inputs to, and losses from, soil. To determine effects on inputs, Russell will measure total annual aboveground crop growth and root biomass production in the top 60 cm of soil in experimental plots established four years ago (Ames, IA) in a corn-soybean rotation under three levels of N fertilization: 60, 120 and 180 kg/ha. She will determine fertilizer effects on tissue quality (C, N and lignin content) and decomposability and soil CO_2 losses to the atmosphere. Her approach will be to integrate field and laboratory experimentation with process-based modeling using the CENTURY model. This topic is highly relevant because, with so much land in the upper Midwest devoted to corn production, nitrogen fertilization effects that result in small annual decreases in SOM will contribute to soil degradation on a large scale, with important consequences to soil fertility, water resource quality and global biogeochemical cycles.

9900997 Grassland Ecosystem Response to the Experimental Manipulation of Precipitation
Knapp, A.K.; Blair, J.M.; Fay, P.A.
Kansas State University; Division of Biology; Manhattan, KS 66506
Grant 99-35101-7898; $253,500; 3 Years

A high degree of climatic variability, particularly in rainfall, both between years and within the growing season is characteristic of all grasslands. Climate change predictions for temperate grassland regions include decreased rainfall amounts and, perhaps more importantly, increased variability in the timing of precipitation events. The tallgrass prairies in Kansas support a livestock grazing industry second only to Texas in stocking rate. Vegetative productivity in these grasslands, which relates directly to livestock production, is strongly influenced by precipitation patterns. Thus, the major goals of this research are to determine how key aboveground ecosystem processes are affected by experimental alterations in the amount and timing of precipitation events. As part of this research, we will identify the potential consequences of these responses for important grassland ecosystem function such as productivity. We will experimentally alter the timing and amounts of rainfall events independently, and in tandem, under field conditions and assess individual plant, community and ecosystem responses. These experiments will be performed on the Konza Prairie Research Natural Area, a Long-Term Ecological Research Site in Northeast Kansas. The results of this multidisciplinary research approach will provide key insights into the importance of alterations in precipitation patterns that are predicted to occur under a variety of climate change scenarios.

9900958 Trade-off Effects of Human Disturbance (Forest Management Practices) on the Structure and Function of Plant-Insect Communities in North Temperate Forests

Woods, S.A.; Stubbs, C.S.; Drummond, F.A.
University of Maine; Department of Biological Sciences; Orono, ME 04469-5722
Grant 99-35101-7747; $213,500; 3 Years

Clearcut harvesting and applications of herbicides represent forestry practices that have a substantial impact on the forest ecosystem and raise considerable controversy. The controversy, however, is often based on aesthetics rather than a science. By increasing plant diversity and the abundance of flowering plants, clearcut harvests may have a positive effect on beneficial insects such as wild bees and parasitic insects that keep insect pest populations in check. Benefits may be observed both in the clearcut areas and adjacent mature forest stands. Conversely, herbicides are used to reduce competition from flowering plants and may diminish any benefits of the harvest. We will study the impact of clearcut harvests and herbicide applications on plant and insect communities. Sites will include both herbicide-treated and untreated clearcuts. Vegetation surveys and insect trapping techniques will be used to evaluate the effects of management within the clearcut areas and into the interior of adjacent mature stands. Emphasis will be placed on the wild bees and parasitic insects because they play beneficial roles and occupy many levels in the food chain; and their forage plants because these plants are the target of herbicide applications. We hypothesize that clearcut harvests will benefit wild bee and wasp communities, but that herbicide applications, at least in the short-term, will reduce the benefits. Results of the research should lead to specific guidelines for management practices that maximize the diversity and the abundance of beneficial insects and forage plants that promote productive, sustainable use of north temperate forests.

9900944 Landscape Scale Interactions Between Fire Regimes and Ecosystems
Miller, C.L.
University of Montana; School of Forestry; Missoula, MT 59812
Postdoctoral Fellowship; Grant 99-35101-7828; $88,000; 2 Years

Fires play an important role in many ecosystems by removing accumulated fuels, creating establishment sites for new plants, enhancing wildlife habitat, increasing water yields, and increasing the availability of soil nutrients. Fire suppression during the past century has contributed to the deterioration of many fire-dependent ecosystems and to the increase in uncontrollable wildfires. Although managers are striving to restore the process of fire to fire-dependent ecosystems, most of our understanding of fire lies in its short term effects and does not extend to the large spatial scales at which managers must manage. An understanding of how fires and ecosystems interact over long time frames and at large spatial scales is necessary if we are to sustainably manage fire-dependent ecosystems. The objective of my research is to provide guidance for fire management and planning through an improved understanding of the interactions among topography, vegetation, and fire. I have three specific objectives: (1) improve how fire regimes are described at large spatial scales, (2) develop a computer model to determine how topography and vegetation influence the occurrence, severity, and spatial distribution of fires, and (3) use the model to investigate the long term interactions between fire and vegetation distributions across landscapes. I will develop a computer model for the Selway-Bitterroot Wilderness Area in northern Idaho and western Montana to simulate fire patterns and vegetation dynamics. Historical fire data will be used to test the model and to evaluate new approaches for describing fire regimes. My goal is to assess the long term consequences of fire management activities on vegetation distributions and fire regimes.

9901082 The Effect of Harvester Ant Nests on Nitrogen Cycling in Arid Rangeland Soils
Wagner, D.; Jones, J.B.
University of Nevada, Las Vegas; Department of Biological Sciences; Las Vegas, NV, 89154-4004
New Investigator/Strengthening Award; Grant 99-35101-7834; $180,000; 3 Years

The goal of the proposed research is to estimate the effects of a common, soil-dwelling animal species, the harvester ant (*Pogonomyrmex barbatus*), on nitrogen cycling in arid rangeland soils. Often viewed as pests because of their seed eating habits and venomous sting, preliminary data suggest that harvester ants play a significant role in nutrient cycling. Ants concentrate organic debris in the vicinity of the nest, thereby creating islands rich in organic matter and mineral soil nutrients within the landscape. On the study site, harvester ant nests occupy only about 1% of the surface area of the landscape but we estimate that ant nest soils contain 9 - 14% of the total available soil nitrogen on the plot. We hypothesize that ant nests alter major N transformations and play an important role in controlling rangeland soil fertility. The influence of harvester ants on nutrient cycling at large spatial scales depends on the density, age and size of nests. The proposed research will combine information about how ants affect soil nutrient levels and nutrient cycling with information about ant population biology to estimate the effects of harvester ant populations on nitrogen cycling at large spatial scales.

9900979 Plant Diversity, Production, and Stability in Grassland Ecosystems

Biondini, M.E.; Grygiel, C.E.
North Dakota State University; Department of Animal and Range Sciences; Fargo, ND 58105
Grant 99-35101-7807; $279,500; 4 Years.

The hypothesis that the function of ecosystems depends on their biological diversity has been part of ecological theory for more than a 100 years. Despite its centrality to natural resource management, knowledge of the precise links between biological diversity, productivity, stability, and sustainability of natural and managed ecosystems is very limited. The problem carries substantial economic implications in mine land reclamation where the achievement of particular diversity targets is mandated by law to show that "the species are abundant enough to demonstrate that permanence and land use capabilities are achieved." Prairie restoration efforts, in particular the Conservation Reserve Program, face a similar problem but on a larger scale. While there are yet no legal standards regarding diversity, productivity, stability, and sustainability, the stated objectives of the program, "the restoration and permanence of prairie ecosystems, the protection of their long term productivity, and the enhancement of wildlife habitats" requires such standards. The proposed research will generate information in 3 areas important to the management of grassland ecosystems: (1) What are the combinations of temporal and spatial scales in plant resource utilization patterns that beget high species diversity and how can they be manipulated to achieve that goal? (2) Does the productivity of an ecosystem depend on species diversity, and if so why? (3) Does the ecosystem stability depend on species diversity, and if so why? The information will provide policy makers with better tools to evaluate how changes in plant biodiversity can affect the long term productivity and sustainability of Great Plains grasslands.

9900975 Ecosystem Consequences of Globalized N Deposition - N Losses to Water and Air

McDowell, W.H.
University of New Hampshire; Department of Natural Resources; Durham, NH 03824
Strengthening Award; Grant 99-35101-8529; $100,000; 2 Years

The sources and distribution of anthropogenic nitrogen (N) are rapidly shifting from concentration in the more-industrialized temperate regions to a more global distribution. However, scientific understanding of the consequences of this anthropogenic nitrogen deposition, such as trace gas emissions and nitrate leaching, is based almost entirely on studies of N-limited systems carried out in the temperate zone. It seems likely that many, tropical ecosystems will respond quite differently to anthropogenic inputs of nitrogen due to differences in nutrient limitation. Tropical forests are hypothesized to be less able to retain anthropogenic nitrogen, and more likely to show immediate N losses in soil solution with increased N deposition. To evaluate this hypothesis, experimental plots will be established in the Luquillo Experimental Forest, Puerto Rico. Changes in soil solution chemistry will be measured in response to elevated N inputs in two forest types. The experimental design consists of replicated plots receiving 150 kg/ha/yr N as NH_4NO_3, and a separate riparian experiment in which losses of N will be measured as shallow groundwater moves through the riparian zone. The results of this research project will have important implications for management and sustainability of tropical landscapes, and will provide fundamental information on the nature of the N retention process in terrestrial landscapes.

9900909 Exploring the Causes of Failed Oak Regeneration in Eastern Deciduous Forest: Test of Four Prominent Hypotheses

Carson, W.P.; Adams, M.B.
University of Pittsburgh; Department of Biological Sciences; Pittsburgh, PA 15260
Grant 99-35101-7732; $199,400; 4 Years

Oaks are the most abundant tree species throughout much of the forests of the eastern United States. Unfortunately many oak forests are not regenerating and appear to be converting to forests dominated by species with less timber value as well as less value to wildlife. Consequently, the US Fish and Wildlife Service has classified four widespread oak ecosystems as endangered. The causes of failed oak regeneration remain poorly understood. Research on failed oak regeneration is critical because eastern forests have lost other economically valuable tree species (e.g., chestnut). To address these issues, we have begun large-scale experiments that are designed to test four hypotheses proposed to explain failed oak regeneration. These include: (1) The fire hypothesis: Periodic fires promote oak regeneration; (2) The browsing hypothesis: Browsing by high populations of deer kills oak saplings; (3) The seed predation hypothesis: Squirrels and other small mammals eat and kill acorns preventing seedling establishment; Finally, (4) The treefall gap hypothesis: Oaks require large treefall gaps (openings in the overstory) to regenerate in the absence of large-scale disturbances. These hypotheses must be tested together because each of the above forces may be operating at the same time. Thus, we are testing them by using controlled burns, excluding deer browsing with fences, cutting down trees to create gaps in the canopy, and using fine mesh fencing to remove seed predators such as squirrels. These experiments are designed to run for 10 years and thus we believe we can discover the causes of failed oak regeneration.

9901080 Isotopic Analyses of Ecosystem Respiration and Photosynthetic Discrimination Along the OTTER Transect

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Grant 99-35101-7772; $385,500; 3 Years

As concerns grow about increases in atmospheric greenhouse gases and potential changes to our global climate, there is increasing attention to the role of forests as potential carbon sinks. However, the potential for forests to sequester carbon dioxide, as well as management options for maximizing sequestration, are poorly quantified. This lack of information is due, in part, to the difficulty of measuring and partitioning carbon fluxes accurately on ecosystem scales. The stable isotope techniques we propose to develop for analyzing isotope discrimination on ecosystem scales are likely provide new and efficient approaches for monitoring the impacts of management options on critical aspects of the carbon cycle. The study will be conducted in four forest types along the Oregon transect (OTTER): spruce hemlock, Douglas fir, ponderosa pine, and juniper. This is a powerful system for this project for several reasons. First, across a short distance, the moisture gradient spans from some of the most mesic to the most xeric climates in North America. Second, extensive research over more than two decades has produced a rich literature about these forests. Finally, there are existing ecosystem-level flux measurement studies underway at two of the sites. Our research will be conducted in close association with the flux measurements to the mutual benefit of both projects.

9901070 Probing the Mechanisms by which Subcanopy Evergreen Shrubs Inhibit Tree Seedling Recruitment
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Grant 99-35101-7778; $489,000; 4 Years

Evergreen shrubs inhibit the recruitment and growth of commercially important canopy tree seedlings in the southern Appalachian forest. In high quality cove forests *R. maximum* inhibits seedling recruitment of important forest trees, and in the severely degraded pine-hardwood forest *K. latifolia* inhibits the recruitment of trees and the reestablishment of a healthy forest. This inhibitory effect of subcanopy shrubs is becoming more important for forest management and the forest industry because the spatial extent of these shrubs is expanding. In order to develop sustainable forest management plans, it is critical to understand the mechanisms by which these evergreen shrubs inhibit canopy tree seedling recruitment. In our previous research, we found that pre-germination factors (seed rain, seed bank, allelopathy) were not important inhibitors of seedling recruitment by *R. maximum*. However, light competition and mycorrhizal inhibition were important. In this project, we plan to validate our suggested model of seedling inhibition, and to expand our study to a broader region of the Appalachian forest including a second shrub species. The outcome of this study will be an extensive data set covering seedling growth, survivorship, physiological performance, and mycorrhization using a natural gradient of light and resource manipulations. With this data we will be able to use established statistical tests to investigate the significance and magnitude of the light-mycorrhizae interaction on seedling growth and survival of two commercially important canopy trees. These data will provide solid basic scientific information for the development of sustainable forest management programs in the southern Appalachian Mountains.

9900995 Forest Site Heterogeneity Dynamics and Their Interactions with the Forest Community
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Grant 99-35101-7872; $241,500; 3 Years

Soil properties and vegetation are heterogeneous; i.e., they are variable in space and time. Studies of this variation, spurred by recent developments in ecological theory and statistics, have provided new insights into plant competition and succession. In this renewal of USDA grant we will: (1) document spatial patterns of soil and vegetation during secondary succession in upland forests of southeastern United States; (2) explore plant effects on spatial variation in soils; (3) determine if soil variability influences plant competition; (4) improve a mathematical model to simulate changes in spatial pattern over time; and (5) use the model to evaluate how different forest management activities may lead to different plant communities. At DOE's Savannah River Site (SRS) in South Carolina, we will sample soil and vegetation in forests of different ages (objective 1), and set up garden plots to examine interactions of soil and vegetation pattern (objectives 2 and 3). At the Virginia Tech campus, we will use greenhouse studies for the study of plant effects on soil (objective 2) and conduct the modeling (objectives 4 and 5). Currently, most studies of this type focus on the responses of organisms to environmental heterogeneity as shown in the recent British Ecological Society Annual Symposium. Our work is unique in its search for changes in spatial pattern over time, and the reasons behind these changes.

9900913 Microbial and Physico-chemical Control of Nitrogen Mobility in Forest Ecosystems
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Strengthening Award; Grant 99-35101-8235; $100,000; 2 Years

Atmospheric deposition of nitrogen on forest ecosystems, from industrial and automotive emissions, has been linked to the acidification of lakes and streams. Understanding what factors control the movement of nitrate through the forest ecosystem is essential to an understanding of the impacts of deposition. Previous work has shown that the nitrate that reaches streams is derived from soil microbial processes, not directly from deposition. This study will focus on the forest soil microbial population and how it transforms nitrogen. Additionally, it will compare a number of established and new techniques to estimate the rate of nitrogen transformations in forest soils of the northeast United States. The stable isotopes of nitrogen and oxygen in nitrate will be measured to determine the source and fate of nitrogen as it moves through the ecosystem. Molecular biology approaches will be used to characterize the microbial community with special attention to the ammonia oxidizing population (the group that produces nitrate). The relationship between the microbial community and the rate of nitrate production will be assessed. Results should provide valuable information on the role of microbial community dynamics in watershed nitrogen mobility and provide insights on the mechanism of increased acidification from increased nitrogen deposition.

9900924 Effect of Cropping Systems on Shrub-steppe Habitat
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Grant 99-35101-7858; $188,000; 3 Years

Grasslands in the western United States are integral components of regional agro-ecosystems, providing refuge for wildlife, enhancing regional biodiversity, and serving as essential reservoirs for biological control agents of crop pests. Reductions in the quality and quantity of grassland habitats may affect the sustainability of regional cropping systems. Agricultural systems have caused extensive fragmentation of grasslands, and have created highly disturbed regional environments through repeated tillage, harvesting, and high agrochemical inputs. The proposed 2 1/2-year project will determine the effect of fragment size and adjacent cropping systems (i.e., annual vs. perennial crops) on the productivity, diversity, and community composition of plants and arthropods within grassland habitats in the Columbia Basin Region of the Pacific Northwest. Sixty shrub-steppe sites will be selected randomly within the region; 20 sites in small fragments (i.e., corners of irrigation circles) associated with annual crops, 20 sites in small fragments associated with perennial crops, and 20 sites within large shrub-steppe habitats. Coverage, diversity, and biomass of annual and perennial plants; and the diversity, abundance, and trophic structure of arthropods will be estimated along transects at each site. Comparisons of plant and arthropod communities in the three habitat types are expected to show that productivity, diversity, and trophic structure change along a continuum of fragment size and disturbance caused by cropping systems. Systems dominated by annual crops are expected to have more of an impact on shrub-steppe communities than those dominated by perennial crops.

PLANT RESPONSES TO THE ENVIRONMENT

Panel Manager - Dr. Justin Roberts, University of California, Riverside
Program Director - Dr. Anne H. Datko

Awards in this area support research aimed at understanding the plant's response to environmental factors, both natural and anthropogenic. The major goal of the program is to provide the basic knowledge needed for devising strategies for decreasing the impact of environmental stress and for adapting agricultural and forest practices to possible changes predicted to accompany global climate fluctuations.

Studies on mechanisms at the whole plant, cellular or molecular level which explain organismal response are emphasized. The environmental factors of interest include water, temperature, light (including UV-B), nutrient, and atmospheric chemical composition (including carbon dioxide and other greenhouse gases, sulfur dioxide and ozone).

9900575 Plant Thermotolerance: The Role of Small Heat Shock Proteins and Other Factors

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Grant 99-35100-7618; $200,000; 3 Years

The long term goal of our research is to define factors that contribute to plant high temperature tolerance. Plants have many different mechanisms for surviving high temperatures in their environment. These include long term evolutionary adaptations of life history and morphology, as well as medium term avoidance or acclimation mechanisms. However, plants can also rapidly adapt to high temperature (acquired thermotolerance) over a period of hours. Even plants growing in optimal environments may experience routine instances of potentially lethal heat stress, and the rapid development of acquired thermotolerance is of significant importance to plants. In addition, because heat stress is most often correlated with water deficit, the ability to survive heat stress probably contributes in many instances to survival under drought conditions. It has proven difficult to increase plant tolerance to high temperature stress by conventional breeding approaches. In order to manipulate high temperature tolerance in whole plants it is important to identify genes that make a major contribution to thermotolerance, either as thermotolerance components or as regulators of these components. Engineering thermotolerance by altering the regulation of such genes or by adding specific thermotolerant proteins to plant genomes should then be possible. Such an approach has recently been used successfully to enhance cold tolerance in the model genetic organism, *Arabidopsis*. We propose a systematic genetic analysis to identify components required for thermotolerance in *Arabidopsis*. Thermotolerance mechanisms identified by this approach should be readily adapted to genetic manipulation of crop plants.

990627 Dry Deposition of Nitric Acid Pollutant: Effects on Foliar Surfaces and the Consequences for Growth and Physiological Activity by Western Forest Trees

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Grant 99-35100-7600; $180,000; 3 Years

Many arid and semi-arid ecosystems are experiencing deposition of nitrogenous air pollutants. This deposition is resulting in measurable changes in plant nutrient availability. In semi-arid environments, wet deposition of pollutants (i.e., acid rain) is rare due to the dry climate. However, deposition of dry pollutants can result in substantial biological, chemical and physical impacts. Most of the deposited pollutants in urban settings occur as nitric acid vapor (HNO_3) or particulates containing nitrate. These pollutants have direct biological effects and indirect fertilizing effects on plants and ecosystems. We propose that nitrogen dry deposition is a contributing factor to the decline of forest ecosystems in southern California. While increased nitrogen fertility may have initially positive effects, such as enhanced growth, the data have shown that chronic exposure may also have negative effects including exacerbated nutrient deficiencies and imbalances, and reduced root growth increasing susceptibility to water stress. However, little is known about the biochemistry and physiology of trees exposed to these airborne pollutants. The existing information indicates that individual plants are capable of absorbing and assimilating nitrogen deposited to foliage but there is little information on the subsequent physiology. We propose three lines of investigation: (1) a detailed investigation of leaf surface interactions with atmospheric HNO_3, (2) seedling growth responses as a result of assimilation of atmospheric HNO_3, and (3) evaluation of the interaction between shoot uptake and root function. These investigations will be carried out in large fumigation chambers that are designed to mimic typical conditions encountered in the pollution-impacted forests of southern California.

9900999 Osmosensing Mechanisms in Guard Cells

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Grant 99-35100-7599; $155,000; 3 Years

Water stress is one of the primary factors limiting crop productivity. During evolution, plants have developed a number of mechanisms to cope with water stress. One of the mechanisms is to close their stomata under drought conditions. Our long term goal is to understand the link between the drought environment to stomatal closure. Previous studies have identified at least two major pathways triggered by water stress, one is initiated by production of abscisic acid (ABA) and the other is an ABA-independent process. While many research efforts have been directed to the ABA pathway, little is understood about the ABA-independent pathway. We have recently established a model system with which we can address the direct osmosensing process of ion channels in guard cells of stomata. The proposed research seeks to combine several different approaches to elucidate the molecular mechanism underlying osmosensing of ion channels in guard cells. Understanding the mechanisms underlying stomatal regulation by osmotic stress will provide important information for engineering crop plants with higher tolerance to drought, which is important for agriculture worldwide.

9900648 Genotypic Adaptation and Phenotypic Acclimation to Elevated CO_2

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Grant 99-35100-7577; $180,000; 3 Years

My research has two long-term goals: (1) to characterize how environmental parameters modulate carbon assimilation, including gene expression, and (2) to identify components that could be manipulated by molecular means to improve crop production efficiency and sustainability in future environments. The specific objective of this study is to document the adaptation and acclimation responses of a plant that has been exposed to high CO_2 in its natural environment. The research focuses on how plants respond to climate change, but it has several novel aspects, such as focusing on a freshwater system. Freshwater is a vital resource, with increasing value as population and agricultural pressures increase, and aquatic plants are an integral component of a healthy ecosystem. This research also focuses on genotypic adaptation, whereas most studies just investigate short-term phenotypic acclimation. We will take advantage of an amphibious plant, *Ludwigia repens,* that has been exposed for generations to high dissolved and atmospheric CO_2 in Florida springs. Seeds will be collected from two genetically isolated populations in springs with low and high concentrations of CO_2, and grown in various CO_2 atmospheric regimes in a controlled outdoor system designed for climate-change research. Growth rates and specific photosynthetic parameters (gas exchange, enzyme content and activity, and gene expression) will be measured to determine how the populations respond and are adapted to high CO_2. Differences between submersed and aerial leaves on the same plant will also be examined. This study should enhance our understanding of how plants cope with the inexorable rise in atmospheric CO_2.

9900638 Functional Analysis of Plant Heat Shock Transcription Factors

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Grant 99-35100-7001; $200,000; 3 Years

The broad objectives of this proposal are to determine the mechanism of induction of heat shock (HS) genes in plants and to explore the role of the newly discovered class B heat shock transcription factors (HSFs) that have little capacity to activate transcription. This family of HSFs is distinct from other organisms and may represent an ancient adaptation to environmental stress especially suited for plants. Our hypothesis is that the class B HSFs act as repressors of HS gene transcription in order to suppress fortuitous expression of the heat shock response under non-stress conditions. Plant HSFs will be over-and under-expressed in transformed *Arabidopsis* plants to determine the role of class A and B HSFs. Transient assays will probe the mechanism of promoter activation or repression using synthetic promoters containing binding sites for LEXA- and GAL4-HSF fusion proteins to see if the B class of HSFs can suppress transcription. In addition, *in vitro* studies will determine the role that cooperativity may play in the binding of class A and B HSFs to the promoter DNA. In an effort to understand the mechanism of transcriptional induction, nuclear run-on assays will determine if RNA polymerase is paused at heat shock promoters under non-stress conditions. Genetic strategies to engineer the stress response rely on a sound understanding of the role of transcription factors that are involved in its regulation. Information related to HSF function may directly benefit efforts to enhance the ability of plants to withstand a broader range of environmental stresses.

9901049 Localization and Function of *VP14* Specific ABA Synthesis in Maize

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Grant 99-35100-7005; $200,000; 3 Years

Because most crop production in the United States is rain-fed, water availability is a major limiting factor affecting crop yield. In the field, crop plants respond to drought conditions by synthesizing a stress hormone, abscisic acid (ABA). ABA regulates biological responses that allow plants to survive periods of water deficit. ABA controls the rate of water movement and loss from the leaves, and is produced in many plant organs including roots and leaves. However, the physiological functions of ABA originating from different sources in the plant is poorly understood. We have isolated a gene from corn (*Vp14*) that regulates ABA synthesis in these tissues. Our analysis of mutant forms of *Vp14* indicate that this gene controls a specific subset of the corn plant's overall ABA biosynthetic capacity. The lack of ABA synthesis in mutant plants strongly affects water movement through leaves of corn seedlings. The goal of this project is to determine where *vp14* specific ABA synthesis is localized in corn seedlings and understand its physiological function. We will pursue two experimental objectives: (1) compare ABA contents of the specific cells in leaves of the *vp14* mutant and normal leaves, and (2) determine the location of the *vp14* specific ABA synthetic activity in maize plants using techniques for localizing gene expression in plant tissues.

9900584 Avoidance of Aluminum Injury in Legumes: Role of Root Border Cells

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Grant 99-35100-7002; $130,000; 2 Years

Soils are composed of aluminum-containing minerals. Processes such as fertilization or acid rain are acidifying both agricultural and natural ecosystems. Under acidic conditions, aluminum is solubilized from soil minerals, and converted to a form that plants can absorb. High levels of aluminum can stunt root growth; however, certain plant species or varieties within species can tolerate aluminum. In this project, we will investigate the role of border cells in protecting roots from aluminum injury. Root border cells are abundant on most plant species, including agronomically important ones like cereals and legumes. They form a population of living, dissociated cells surrounding the root tip, and they are known to release chemical compounds such as polysaccharides or organic acids that could bind to and detoxify aluminum. We plan to examine three mechanisms by which root border cells could protect the root tip from aluminum injury: (1) exudation of aluminum-complexing organic acids, (2) exudation of aluminum-binding mucilages, and (3) absorption and sequestering of aluminum into the border cells themselves. Three species of legumes will be used in this study, including a model legume whose genome is being sequenced in a separate project. The long-term goal of this study will be to identify genes controlling aluminum tolerance and then to manipulate expression of such genes in genetically modified plants to increase the ability of legumes to resist aluminum injury.

9900622 Sulfur Stress Signal Transduction

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New Investigator Award; Grant 99-35100-7579; $200,000; 3 Years

An adequate supply of nutrients is required for maximum crop productivity and quality. However, agricultural lands are often deficient in one or more of the essential nutrients. Plants can sense their nutrient status and acclimate to nutrient changes in nutrient availability, but plant growth and quality are often diminished. We are studying how photosynthetic organisms sense and acclimate to sulfur deficient conditions. Sulfur is an essential nutrient and is a component of proteins, lipids, carbohydrates, electron transport chains and numerous intermediate metabolites. Plants preferentially import sulfur as the sulfate ion. However, sulfate supplies in the soil can be low even when other forms of sulfur are plentiful. To understand how photosynthetic organisms sense and respond to sulfate deficient conditions, we have been studying the unicellular green alga *Chlamydomonas reinhardtii*. This organism is related to vascular plants but is easier to work with in the laboratory. It also has some enzymes, absent in vascular plants, that enable it to access alternative forms of sulfur when sulfate is not available. We have isolated, characterized and complemented mutants of *Chlamydomonas* that do not acclimate to sulfate deficient conditions. These mutants have genes (already identified) that enable *Chlamydomonas* to sense sulfate levels and/or control its responses to sulfate deficiency. These genes will enable us to understand these processes at the biochemical level. This research may provide us with tools for early detection of sulfur deficient conditions and enable us to understand how plant productivity is limited by sulfur deficiency.

9900608 Glutathione Synthesis in Plants

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Grant 99-35100-7545; $200,000; 3 Years

Crop yields are usually less than half of the maximum capacity of the plants due to non-ideal growing conditions. We lump these non-ideal conditions together as environmental stress. Plants have evolved sophisticated mechanisms to minimize the effects of stress on their growth and reproduction. One of the key chemicals responsible for ameliorating the effects of environmental stress is suggested to be glutathione. In this proposal we will do two groups of experiments. The first group will be to test a number of plants where we have increased and decreased the glutathione levels by genetic engineering techniques. We will be able then to determine the role that glutathione plays in keeping plants healthy. The second group of experiments is designed to see how plants control the level of glutathione in response to toxic heavy metals in the soil. Specifically, we will determine how plants recognize toxic metals (cadmium and copper) in their environment and how they switch on the genes for glutathione synthesis in order to protect the plants from this stress. By studying this natural mechanism by which plants protect themselves from environmental stresses, it should be possible for us to augment these mechanisms in order to produce crops that are more stress resistant and, as a result, higher yielding.

9900589 The Signal Transduction Pathway for Response to Altered CO_2

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Grant 99-35100-7569; $110,000; 2 Years

The long term objective of the proposed research is to understand the pathway in plants that leads to changes in gene expression in response to a change in carbon dioxide (CO_2) availability. Because of increasing atmospheric CO_2 and its potential impact on growth and productivity of plants, the importance of understanding plant responses to changing CO_2 has become critical. Although many plants respond to increased CO_2 with decreased expression of photosynthesis-associated genes, both the perception of altered CO_2 and the pathway converting that "signal" to a response are difficult to study. This is because the changing CO_2 concentration is perceived via metabolic changes and generates complex responses in the plant. Thus, acclimation of the single-celled alga *Chlamydomonas* to CO_2 concentration changes was chosen as a simpler model system for study of CO_2-regulated gene expression. *Chlamydomonas* exhibits a variety of responses to changing CO_2, including specific changes in gene expression. We are investigating the response pathway for adaptation of *Chlamydomonas* to changes in CO_2 by using an induced gene, PCA, as a reporter for action of the response pathway. *Chlamydomonas* mutants unable to respond to CO_2 changes, identified as lacking expression of the reporter gene (PCA), have been generated and are being used to identify components of the response pathway. The information gained from this simple model system should help us to understand and to lay the foundation for further investigations of the responses of more complex plants to increasing CO_2 concentrations, including the potential for modification of the response in crop plants.

9900592 Symposium on Metabolic Networking in Plants

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Grant 99-35100-7586; $5,000; 3 Years

The symposium “Metabolic Networking in Plants” to be held April 22-25, 1999 at Iowa State University, will focus on the interconnections between the pathways that plants use to create and reuse the myriad of chemicals that they contain. Sub-themes are the use of functional genomics in metabolic analyses; impact of environment and development on interconnections between metabolic pathways; the regulation of metabolic pathways; and novel approaches to computer modeling how plant metabolic pathways are integrated. The atmosphere will be informal, and the format allows for extensive discussion both during and between sessions. An important purpose of these symposia is to promote interactions and new collaborations between individuals who might not normally come together at a single symposium. In doing this, we will include a spectra of attendees ranging from world famous scientists to highly promising students and post-doctoral fellows.

As we approach the time that the complete DNA sequence of a plant has been determined, we need to use this wealth of information for defining the intricate metabolic networks. Even if we know the DNA sequence of a gene, we many times do not know its function. We thus need to undertake the task of assigning metabolic functions to batteries of genes on a large scale, identify interrelations among different metabolic pathways and deciphering the interplay in their modulation by environmental and developmental cues. We see the symposium as a way to educate students and post-doctoral associates and to promote meaningful collaborations and interdisciplinary interactions among established scientists.

9900647 Stress Responses of Fermentative and Glycolytic Genes in *Arabidopsis*

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Grant 99-35100-7597; $50,000; 1 Year

Exposure to oxygen deprivation (anoxia) and decreased oxygen availability (hypoxia) due to flooding are common environment stresses encountered by plants. Most crop plants, including barley, maize and wheat, can only tolerate transient flooding. In contrast, rice plants can survive much longer under flooding. Understanding the molecular and cellular mechanisms of how plants respond to low-oxygen stress is essential in our efforts in developing crop plants with improved flooding tolerance. It has been shown previously that carbon metabolism of plant cells switches from aerobic respiration to fermentation after a change from aerobic to hypoxic/anoxic conditions. This switch results in the induction of the alcohol dehydrogenase gene (*ADH*) and other glycolytic genes in root cells of maize and *Arabidopsis*. The main goal of this proposal is to identify signaling events involved in the induction of these genes during hypoxia and anoxia in *Arabidopsis*. We have used a genetic approach to isolate a total of 18 mutants, designated as *aar*, which are defective in anoxic and hypoxic induction of *ADH* and glycolytic genes. In the time period covered by this proposal, a series of biochemical and molecular experiments is proposed to characterize these *aar* mutants. Results from the proposed experiments should allow us to determine the number of genes that are affected in these mutants and which regulatory steps are affected by these mutations. The information obtained will help elucidate the signaling pathways leading to the induction of fermentative and glycolytic genes during hypoxia and anoxia in plants.

9900602. Calcium-binding Properties of Cold-and Dehydration-stress Related Proteins

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Grant 99-35100-7668; $180,000; 3 Years

Sudden exposure to environmental stress is a major problem for agriculture in temperate parts of the world, resulting in catastrophic economic and productivity losses. Some plants have the ability to develop a tolerance to chilling and dehydration injury, and some can develop a tolerance to freezing injury. Initial plant responses to low temperature stress includes the elevation of cellular calcium levels; and a sustained exposure to cold stress can result in chronic calcium elevation (resulting in cell death). Many studies indicate that control of calcium levels is critical for environmental sensing and for maintaining plant growth. Among the many responses of plants to low temperature stress and dehydration is an increase in dehydrin proteins. The function of these proteins is currently unknown. Our preliminary evidence strongly suggests that the role of some of the dehydrins is related to calcium binding. This connection between stress-induced increases in cellular calcium and the cold induction of calcium-binding proteins suggests a physiological role for these proteins in cold stress management. We will test the hypothesis that dehydrins bind calcium, and study dehydrin proteins from celery and the genetic model system, *Arabidopsis*. A further determination of the location of these proteins within the cell and the mechanisms by which their calcium-binding properties may be regulated will allow us to understand the biochemical mechanisms that underlie plant responses to cold and dehydration stress. Through this increased understanding we will be able to better manage plants in variable and adverse environments.

9900621 Molecular and Genetic Analysis of the Role of the Maize *ys1* Gene in Iron Uptake.

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Grant 99-35100-7601; $120,000; 2 Years

Adequate iron uptake by crop plants is necessary for optimizing crop yields and for ensuring that edible crops contain adequate amounts of iron for the diets of both humans and livestock. Iron nutritional status of crop plants is determined largely by the efficiency with which they can take up iron from the soil. The Poaceae (grass family), which are among the world's most important crop plants (e.g., rice, maize, and wheat), take up iron by a mechanism that is fundamentally different from that of other plant species. This project constitutes the first examination of the process of iron uptake by grasses at the molecular level. The product of the yellow stripe1 gene (*Ys1*) of maize is involved in mediating the up take of iron bound to phytosiderophores, a class of iron-solubilizing compounds that are secreted by grasses in response to iron starvation. With a *ys1* clone now in hand, we will conduct studies that will elucidate the precise role of *Ys1* in iron uptake. We will determine the locations in the plant where *Ys1* is expressed to determine whether it is involved in primary uptake or in transport to tissues. We will examine whether the YS1 protein is a transporter of iron, as expected from physiological experiments, and if so, what compound(s) it is capable of transporting. We will examine how expression of *Ys1* is controlled by the iron nutrition status of the plant. The results from these experiments will elucidate the process by which graminaceous plants use the phytosiderophores they secrete.

9900605 Mechanism of CBF Gene Regulation by Low Temperature

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Grant 99-35100-6999; $290,000; 3 Years

It is estimated that the major row crops in the United States yield only about 20% of their genetic potential on an annual basis and that the large majority of this shortfall is due to adverse environmental conditions such as extremes in temperature and drought. Determining the mechanisms that plants have evolved to survive environmental stresses is not only fundamental to a basic understanding of plant growth and development, but also has the potential to provide novel approaches for improving the environmental stress tolerance of agronomic plants. Our primary interest is to understand the mechanisms that plants have evolved to survive freezing temperatures. Toward this end, we recently identified three transcriptional activators in *Arabidopsis*—CBF1, CBF2 and CBF3—that act as "master-switches" controlling the expression of a regulon of freezing tolerance genes. Significantly, we have found that the *CBF* genes are rapidly induced in response to low temperature. Our goal now is to determine the mechanism of this regulation. Promoter fusion studies indicate that low temperature-induction of the *CBF* genes occurs at least in part at the transcriptional level. The specific objectives of this project are to identify the cold responsive element(s) present the *CBF* promoters; determine the nature of the transcription factor(s) that interacts with the element(s); and begin to determine the nature of the signal transduction pathway that activates expression of the *CBF* genes. Knowledge gained from these studies may suggest strategies to modify the expression of *CBF* regulons in plants and thereby improve plant freezing tolerance.

9900617 Resolving Cell Dynamics of Calcium, pH and Endomembrane Responses to Signals and Stressors

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Grant 99-35100-7003; $110,000; 2 Years

Environmentally imposed stresses create huge crop losses. Many environmental stresses shift the levels of calcium in plant cells, and these shifts are proven in a number of cases to mediate protective responses. However, it is unclear how a cell can discriminate calcium shifts due to different environmental stresses and thus produce the required specificity of stress response. We propose to take a step toward understanding specificity by imaging with a fluorescence microscope that has been developed at Washington University to study the temporal-spatial patterns of calcium shifts at high resolution. Such work is possible because another lab has developed a new, relatively accurate and noninvasive, flourescent calcium concentration reporter protein that may be transiently or permanently expressed in the cytoplasm or other "compartments" of plant cells. After appropriately expressing the calcium reporter in a selected cell type, we will apply selected stresses while capturing the 3-D "movie" images. We will place emphasis on mapping concentration shifts in that volume of cytoplasm between the cell membrane and a subjacent region containing endomembrane, because these layers of membrane control much of the flow of calcium in and out of the cytoplasm. We will then evaluate possible relationships between the kinds of stress applied and the temporal-spatial patterns observed. To the extent that it can be discovered how information is encoded during calcium signaling, it may prove possible to manipulate signaling in plants to help them adapt and even pre-adapt to specific stressors as they occur or are anticipated in the field.

9901005 Genetic Analysis of the Oxidative Stress Response in a Cyanobacterium

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Grant 99-35100-7582; $150,000; 3 Years

Major environmental stresses that affect plants are radiation, light, temperature fluctuations, dehydration and chemicals. One way in which several of these stress agents affect plants is through chemically active forms of oxygen, called reactive oxygen species. This is also called oxidative stress, a process that can cause plant crop damage. The reactive oxygen species are even produced in plants by normal, essential processes, such as photosynthesis. Because reactive oxygen species cause many types of damage to plant cells it is important to understand how plants recognize oxidative stress and how they overcome it. There are several important questions to be answered: How do cells sense oxidation? How are known mechanisms to combat oxidative stress regulated in the plant cell? Are there as yet unknown mechanisms to combat oxidative stress? A model cyanobacterial system will be studied to begin to answer these questions. Cyanobacteria share many characteristics with higher plants, but have the advantage that they can be more easily studied genetically. This project will involve a molecular genetic approach to identifying the genes that are important to the survival of cells exposed to agents that produce reactive oxygen species and induce the oxidative stress response. Understanding the cell's response to oxidative damage is the first step to developing methods to protect plants from this injury and to prevent crop damage.

9900598 Regulation of Metal Uptake in Plants

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Postdoctoral Fellowship; Grant 99-35100-7762; $90,000; 2 Years

Many metals, like iron and zinc, are essential nutrients, and the ability to accumulate these nutrients from the diet is crucial for human health. Currently, more people suffer from malnutrition due to micronutrient deficiencies than from deficiencies of protein or energy and it is estimated that 2.7 billion people worldwide suffer from iron deficiency (http://www.who.int/nut/malnutrition_ worldwide.htm#ida). In agriculture, the availability of metals in soil plays a major part in determining crop yields. In addition, plants are the main dietary source of these essential metals for much of the world's population. However, metals can also be toxic to both plants and animals when they are present at high levels. Thus, all cells must carefully regulate their metal levels. In particular, the transport of metals into the cell is a primary control point for the regulation of metal levels. The long range goal of these studies is to understand how cells regulate the uptake of metal ions. The central hypothesis of this research is that a novel group of proteins named the ZIP family (for ZRT-like and IRT-like Protein) plays an important role in the uptake of metals by cells. Specifically, I will examine the regulation of two ZIP transporter proteins, IRT1 and ZIP3. The proposed studies will ultimately lead to a better understanding of metal uptake in plants and to the design of plants that are capable of growth on micronutrient deficient soils and that accumulate metals like iron and zinc and thus have a higher nutritional value.

9900594 L-Ascorbic Acid Biosynthesis and Its Role in Oxidative Stress Protection

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Grant 99-35100-7000; $290,000; 3 Years

Highly damaging reactive oxygen species are generated in aerobic organisms by metabolic processes, xenobiotic agents and in response to pathogens. They are produced in plants by a variety of environmental factors including air pollutants, high light combined with extreme temperatures, drought and pathogens and can lead to decreased crop yield. Plants possess several antioxidants that detoxify reactive oxygen species. One of the most powerful of these is L-ascorbic acid (Vitamin C or AsA). AsA is an essential nutrient in the human diet and has many roles including a protective effect against high blood pressure.

Only recently has progress been made towards understanding how plants make AsA. AsA-deficient mutants of the *Arabidopsis* plant are being used to identify genes (named *VTC* for Vitamin C) involved in AsA biosynthesis. The gene *VTC1* encodes an enzyme in a recently proposed AsA biosynthetic pathway. Two additional *VTC* genes will be isolated during this funding period. Little is known about how AsA biosynthesis is regulated and where AsA is made within the plant. These areas will be investigated using the *VTC1* gene as a tool. The enzyme encoded by *VTC1*, as well as a second known plant AsA biosynthetic enzyme will be overproduced in transgenic plants in an attempt to increase intracellular AsA. Such plants, as well as plants pre-fed with AsA will be tested for enhanced resistance to environmental sources of reactive oxygen. Such resistance could result in substantial agronomic benefits. In addition, transgenic plants with elevated AsA would have increased nutritive value.

9900583 Engineering Improved Environmental Stress Tolerance in Higher Plants

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Grant 99-35100-7004; $150,000; 3 Years

Basic knowledge about the fundamental mechanisms of plant responses to abiotic stresses including salinity, drought, and freezing stresses are crucial to future agricultural productivity. Bacteria, algae, and plants have evolved a common set of adaptive responses to ameliorate the damaging effects of dehydration, salinity, and cold stress. These adaptations include the accumulation of low molecular weight compounds known collectively as compatible solutes or osmoprotectants and glycine-rich, hydrophilic proteins known as late embryogenesis abundant (LEA) proteins. To determine the role of the Group I and II LEA proteins, transgenic *Arabidopsis* plants have been generated that over express these proteins and show improved growth performance under salinity stress conditions. However, the exact mechanisms by which LEA proteins confer stress tolerance are poorly understood. To improve our understanding of how LEA proteins confer stress tolerance, we propose to quantitatively evaluate the growth performance of LEA-overexpressing *Arabidopsis* plants under dehydration, salinity, and cold stress conditions. Large amounts of these LEA proteins will also be overexpressed and purified from *E. coli*. The structure, function, and biophysical properties of these purified LEA proteins will be studied to provide new insights into the mechanisms by which they confer stress protection. Useful strategies for engineering stress tolerance will ultimately depend upon the expression of multiple transgenes. Thus, the long-term goal of this research is to demonstrate the utility of LEA proteins in combinatorial engineering strategies for improving environmental stress tolerance in plants.

9900632 Regulation of Adventitious Root Formation by Soil Phosphorus Availability

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Grant 99-35100-7596; $200,000; 3 Years

Intensive use of phosphorus fertilizers is a major cost to United States agriculture and is a significant source of environmental pollution. In developing countries low availability of phosphorus fertilizers constrains food production. The overall goal of this project is to understand how plants can acquire phosphorus from the soil, so that more efficient crops may be bred that are less costly to grow, more productive, and more environmentally friendly. The specific goal of this project is to understand how phosphorus regulates root growth, and in particular, the formation of adventitious roots. We have observed that phosphorus availability regulates adventitious root formation in common bean in the field and in controlled environments. The ability to form adventitious roots under phosphorus stress appears to be associated with genetic differences in adaptation to low phosphorus soil. This project will explore the functional implications, physiological mechanisms, and genetic basis of this phenomenon. We will test the hypothesis that increased adventitious rooting, leading to a shallower root system, improves phosphorus acquisition efficiency in common bean. Physiological studies will test the hypothesis that low phosphorus increases ethylene production, which in turn induces adventitious rooting via increased auxin sensitivity. This project would provide new insights into the architectural basis for nutrient acquisition efficiency by root systems, physiological coordination of root responses to soil conditions, and quantitative tools for the analysis of root architecture. These would contribute to the development of nutrient-efficient crops, which would enhance the efficiency, competitiveness, and long-term viability of US agriculture.

9900595 Physiological Function of Maize Chloroplast Protein Synthesis Elongation Factor EF-Tu in Possible Relation to Thermotolerance.

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Strengthening Award; Grant 99-35100-8550; $100,000; 2 Years

The objective of this research is to understand the mechanism of action of maize chloroplast protein synthesis elongation factor EF-Tu (EF-Tu) in possible relation to thermotolerance. Previous work has shown that a heat tolerant maize line, ZPBL 1304, synthesizes increased amounts of EF-Tu under heat stress conditions, and that the chloroplasts of this line display increased thermostability. The increased synthesis of ZPBL 1304 EF-Tu associates with the heat tolerance phenotype, and in bacteria EF-Tu acts as a molecular chaperone (increases the refolding of unfolded proteins, protects proteins from thermal damage, forms complexes with unfolded proteins) and plays an important role in heat tolerance. Bacterial and maize EF-Tu-s are strikingly similar, and it is possible that EF-Tu in ZPBL 1304 line confers heat tolerance by acting as a molecular chaperone. The work described in this proposal is designed to test this hypothesis. Studies will be conducted to investigate whether EF-Tu acts as a molecular chaperone and helps chloroplasts and/or other cell proteins withstand high temperatures. The effects of EF-Tu on thermostability of enzymes and chloroplast proteins, and the effects of EF-Tu on thermal damage of cell extracts will be examined. In addition, the steady state levels of EF-Tu mRNA will also be measured under normal and heat stress conditions to determine if EF-Tu may be regulated at the level of transcription. The studies outlined in this proposal will provide additional information on the fundamental role of maize EF-Tu. Our results will be of importance in creating new maize strains with greater heat tolerance.

9901006 Testing Transgenic Cotton with Elevated Anti-oxidant Enzymes

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Grant 99-35100-7630; $200,000; 3 Years

On the Texas High Plains, where 20% of the nations cotton is produced, morning temperatures are well below optimum for photosynthesis. Exposure to full sunlight at these chilling temperatures results in the absorption of considerable light energy in excess of that needed for photosynthetic CO_2 assimilation. The absorption of excess light can lead to a profound reduction in photosynthetic capacity that requires several hours in optimum conditions to recover. We developed transgenic cotton plants with elevated chloroplastic superoxide dismutase, ascorbate peroxidase, and glutathione reductase, which exhibit some protection of photosynthesis when exposed to similar conditions in the laboratory. Using these genotypes, our goals are: (a) to elucidate the mechanism that affords protection to the transgenic plants; (b) to determine the limitations to this approach to protecting photosynthesis in cotton; and (c) to determine the performance of these transgenic plants in the field under irrigated and dryland conditions. Our investigations will identify the most sensitive sites of the photosynthetic apparatus of cotton to chilling/high light stress, and assess the interaction between antioxidants and other protective processes. Various aspects of photosynthetic function, as well as growth, cotton fiber yield and quality will be assessed from field-grown populations of transgenic and wild-type plants. These studies of the performance of transgenic

cotton genotypes grown in the field in West Texas will provide the basis for a realistic assessment of the efficacy of this approach to improving crop performance.

9900643 Tenth International Symposium on Iron Nutrition and Interactions in Plants
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USDA Agricultural Research Service; Children's Nutrition Research Center; Houston, TX 77030
Grant 99-35100-7591; $8,389; 1 Year

This grant provides partial support for the 10th International Symposium on Iron Nutrition and Interactions in Plants, to be held in Houston, Texas, May 14 - 19, 2000. The objective of this meeting is to bring together scientists from a broad range of disciplines, in order to provide a forum for researchers to exchange new knowledge, ideas and techniques, and to provide stimulus for future collaborations. Plant iron nutrition is an important field that has relevance not only to crop health and productivity, but also to the nutritional content of harvested foods for human and animal consumption. Plant iron deficiency, in particular, is a national and international concern, because of the numerous soil types and soil conditions which limit iron availability. Because a plant's ability to respond to this environmental stress can involve a number of physical and biological processes, this continuing Symposium in previous years has attracted an international audience of soil scientists, microbiologists, agronomists, plant physiologists, biochemists, molecular biologists, breeders, ecologists, and human nutritionists. In keeping with this range of interests, the scientific program for the 10th International Symposium will span the soil -- microbial -- plant -- human continuum, in order to foster connections and understanding between processes occurring at various physical and biological levels. Both oral and poster presentations will be integrated to educate and challenge the attendees. The outcome of this Symposium will be to enhance our collective knowledge of plant iron nutrition, and the ways in which plants can respond to iron-deficiency stress.

SOILS AND SOIL BIOLOGY

Panel Manager - Dr. Gary Pierzynski, Kansas State University
Program Director - Dr. Michael P. O'Neill

The Soils and Soil Biology Program supports research that will further our understanding of the basic mechanisms contributing to the immense diversity in soil chemical, physical and biological characteristics in both managed and unmanaged soils and sediments. The program was developed in recognition that soils provide the interface between the biotic and abiotic components of terrestrial ecosystems. It is in the soil that many of the essentials for the production of biomass are obtained and here that nutrients from decaying biomass are recycled into usable forms.

9900723 Molecular Simulation of Adsorption at the Clay Mineral/Solution Interface
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Grant 99-35107-7782; $288,500; 2 Years

In soils, the physicochemical behavior and bioavailability of solutes such as plant nutrients and pesticides are controlled largely by their interactions with clay mineral surfaces and dissolved humic and fulvic substances, but the exact nature of these interactions remains obscure, which limits our ability to accurately predict solute movement in soils. In an attempt to elucidate the general molecular mechanisms that govern these solute/mineral interactions, we will conduct molecular dynamic simulations of the adsorption of both inorganic and organic solutes by a variety of idealized soil clay minerals. The simulations will be performed using commercially available molecular mechanics software in conjunction with a valence-type force field which we parameterized to allow us to perform the first ever fully dynamic simulation of clay minerals. In particular, we propose to simulate inorganic cation exchange on montmorillonite and vermiculite, adsorption of a variety of pesticides and hypothetical fulvic acid molecules by kaolinite, montmorillonite, and vermiculite, and adsorption of pesticides by fulvic acid/clay complexes. Furthermore, we propose to use the results of these simulations to calculate such macroscopic parameters of interest as cation exchange selectivities and partition coefficients. These types of parameters can be used in field-scale models that predict solute movement through soils. In this way, the simulations which we propose to perform will help soil managers maintain soil productivity while protecting soil and water quality.

9900717 Conditionally Dispensable Chromosomes in Fungi: A Source of Genes for Rhizosphere Colonization?
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Grant 99-35107-7748; $235,500; 3 Years

Although microorganisms living in the immediate vicinity of the roots (the rhizosphere) can have large effects on the health of plants and the soil they inhabit, little is known about the specific genetic traits that control this community structure. The goal of this research is to characterize the genes in a soil-borne fungus that define its capacity to survive and compete within the rhizosphere. The experimental system involves highly evolved plant-microbe relationships in which the presence of "conditionally dispensable" (CD) chromosomes in the fungus, *Nectria haematococca*, is linked to its capacity to live on or within root systems of a particular plant species. These interactions range from prolific growth of the fungus in the rhizosphere without deleterious effects on root function to an invasive pathogenesis of the root *per se*. The nature of these interactions is at least in part dictated by the interplay of products of the habitat-defining CD chromosome with chemicals from plant roots. For example, one CD chromosome gene product (Hut) can specifically metabolize a naturally occurring amino acid present at high levels in pea root exudates and another (Mak) can specifically detoxify plant-synthesized antibiotics released by roots. The goal of this proposal is to systematically evaluate the contribution of HUT, MAK and potentially other CD-chromosome encoded genes to the rhizosphere competitiveness of *Nectria haematococca*. Identifying the genes for rhizosphere colonization along with the plant's counterparts could provide the tools to manipulate rhizosphere populations to benefit the health of plants and the soil they inhabit.

9901058 Generation, Transport and Transformation of Pesticide Residues on Airborne Fine Particulate Matter Derived from Managed Soils
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Grant 99-35107-7860; $286,500; 3 Years

Federal air regulations are increasingly affecting United States agriculture; examples include the Clean Air Act Amendments of 1990, which classified many pesticides as hazardous air pollutants, and the recent EPA particulate standards, which regulate fine particles for the first time. Given these regulations, it seems likely that agricultural operations which generate pesticide-containing particulate matter will come under scrutiny in the future. The proposed study will be the first to quantify the loss of pesticides bound to fine particulate matter that becomes airborne during tillage-induced soil

erosion. Examining how soil and pesticide properties affect the pesticide-dust loss from the soil surface will delineate the range of conditions under which this loss mechanism is significant. Because subsequent transformation processes may mitigate the hazard posed by particle-bound pesticides leaving agricultural fields, the study will also quantify the sunlight-induced degradation and gas-phase attachment/detachment reactions of pesticides on dust particles generated from a range of soils.

The major goals of the proposed research are to: (1) quantify the loss of particle-bound pesticides from agricultural fields during tillage operations under real-world field conditions; (2) determine the significance of this mechanism as a source for atmospheric pesticides and a sink for applied pesticide; (3) develop a framework so that the loss of dust-bound pesticides from managed fields can be estimated for a wide range of pesticide and soil conditions; and (4) measure the photolysis rate and attachment/detachment of pesticides from dust as a function of soil and pesticide properties in order to estimate the lifetimes and transport distances for pesticides attached to soil-dust particles.

9900856 The Influence of Understory Vegetation on Soil Organic Matter Dynamics in California Forest Plantations
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Grant 99-35107-7842; $231,000; 3 Years

The growing emphasis on plantation forestry as a primary source of forest products requires that forest managers maximize yield through intensive management and frequent rotations. Careful stewardship of soil quality is thus critical to ensure long-term sustainable yields. Removal of understory vegetation and non-commercial tree species to facilitate replanting and increase tree yield is a common practice to reduce competition for moisture and nutrients. However, recent research suggests that changes in litter composition due to understory removal may lead to a decrease in soil quality. Our research will address this problem through a long-term field investigation of the influence of litter quality (mixed-species vs. single-species litters) on soil organic matter (SOM) dynamics in ponderosa pine plantations. We will study plantations in northern California that are managed by the USDA Forest Service. Our central hypothesis is that mixed-species litters have a beneficial influence on the decomposer community (soil microorganisms and fauna) resulting in enhanced soil quality by increasing the amount of SOM and its nutrient content. Our approach will be to carry out a 2-year incubation of single and mixed-species litter labeled with stable isotope tracers (^{13}C and ^{15}N) in plantations with and without understory. The isotope tracers in conjunction with chemical and biological analyses of SOM constituents will be used to follow quantitative changes in microbial populations and nutrients in the litter and SOM. Our overall objective is to apply our data to the development of practicable strategies to preserve soil quality and enhance productivity of forest plantations.

9900767 Molecular Population Genetics of Arbuscular Mycorrhizal Fungi
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Grant 99-35107-7758; $134,500; 2 Years

Arbuscular mycorrhizal fungi (Glomales) colonize roots of plants from 95% of families, including almost all crop species. They facilitate plant mineral nutrition, and confer tolerance to pathogens, drought, salinity, and metal toxicity. Nevertheless, Glomales have largely been ignored as a component of agronomic practices mostly because of our lack of understanding of the basic biology and genetics of these obligate symbionts. This proposal uses molecular population genetics and cytology/developmental biology to determine how genes are transmitted in Glomales. In order to understand whether arbuscular mycorrhizal fungi reproduce clonally or by recombination, we will address a number of fundamental facts in their biology and develop tools needed to further the genetics in these organisms, including: (1) optimizing PCR amplification of genetic libraries for individual spores, (2) describing polymorphic genes (genetic markers) and PCR primers for their amplification from the libraries, (3) using the markers to determine if nuclei within an individual are genetically identical or different, (4) establishing a time course of nuclear behavior during the fungal life cycle, (5) developing *in situ* hybridization markers to nuclei in germinating spores, (6) using the markers to determine the reproductive mode in natural *Glomus* isolates, and (7) analyzing possible genetic isolation between natural populations. Systematic assessment of whether Glomales are clonal or recombining will provide research tools to perform controlled experiments permitting full evaluation of their role in agrosystems.

9900863 Charge-transfer Complexation of Aromatic Compounds with Soil Organic Matter
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Strengthening Award; Grant 99-35107-7816; $243,000; 3 Years

π-π charge-transfer interaction refers to the weak attractive force between electron rich and electron poor aromatic rings. This type of bond has received little attention in the soils and environmental literature, despite indications that it probably occurs in the absorption of some compounds to soil organic matter (SOM). Many synthetic chemicals, including agri-chemicals, can serve as strong π-donors or π-acceptors in π-π charge-transfer complexes. In addition, humic

macromolecules that make up SOM contain subunits that can act as strong π-donors or π-acceptors. The objectives of this research are, first, to determine the thermodynamics of complexation between select donor and acceptor compounds with model compounds representing potential acceptor and donor sites in SOM. The purpose will be to establish structure-reactivity relationships useful for interpreting sorption data. Second, to conduct adsorption experiments on soils, humic materials, and model sorbents that represent SOM (i.e., lignin) in order to establish π-π charge-transfer complexation in the solid state. Complexation will be demonstrated through: (1) chemically manipulating the donor/acceptor properties of the solid, (2) showing deviation of the expected sorption of reference compounds, and (3) obtaining spectroscopic evidence with dissolved humic substances. The donor/acceptor properties of SOM will be parameterized and correlated with functional group concentrations. The focus of this research is relevant to agriculture and other activities that result in contact of chemicals with soil. The output of this research will be a better understanding of the molecular-level interactions of chemicals with soils and the means to predict sorption with greater accuracy.

9900751 The Influence of Aging and Competitive Sorption on the Stabilization of Metals *via* Surface Precipitation in Soils

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Grant 99-35107-7734; $330,000; 3 Years

Contamination of soils with toxic metals derived from industrial, municipal, and agricultural sources is a worldwide concern. Pollution assessment and remediation efforts represent a significant financial burden for agriculture, industry, and government. Frequently, remedial actions are based on the total contaminant metal burden because it is difficult to assess the speciation (form) of a contaminant in soils. The absence of fundamental data describing metal speciation in soils and the mechanisms by which metals are retained in soils over long time periods may lead to inaccurate predictions concerning contamination risks.

Recent research has shown that metals such as nickel and zinc are stabilized on soil minerals through formation of surface precipitates. The degree of stabilization increases greatly with aging. However, limited data are available to assess the generality of this reaction process for metals in soils. Specifically, it is not known if other metals of agricultural and industrial importance are subject to this sequestration process, and if competitive sorption to multiple soil components will limit the formation of surface precipitates.

This research will investigate the effect of environmental factors (pH, metal concentration, and time), and iron oxide and organic matter content on metal surface precipitate formation and metal stabilization. Molecular scale techniques will be used to characterize the surface precipitates and their association with specific soil components. The research will aid in evaluating the risk of metal amendments to agricultural soils *via* sludge and animal waste applications and in soil remediation.

9900756 Impacts of CO and H_2 Production by Legumes on Soil-Atmosphere Trace Gas Exchange

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Strengthening Award; Grant 99-35107-8552; $90,000; 2 Years

The nodules of nitrogen-fixing plants (legumes) produce hydrogen gas, which is emitted to the atmosphere. Nodules are also capable of producing and emitting carbon monoxide (CO). CO is an especially important trace gas that plays a major role in atmospheric chemistry. Due to their broad distribution, their importance in agriculture and potential rates of CO production, legumes may have a significant impact on exchanges of hydrogen and CO between soils and the atmosphere. In addition, emission of both hydrogen and CO by nodules can affect rhizosphere microbiology by enhancing populations of trace-gas-consuming bacteria.

Since the mechanisms for CO production by nodules are unknown, we are undertaking an effort to determine the sources of nodule CO and controls of emission. Our research will investigate the potential for CO production by legume bacterial symbionts (e.g., *Rhizobium* spp.) *via* a side reaction of the enzyme, nitrogenase, or *via* degradation of the oxygen-carrying protein, leghaemoglobin. These assays will involve pure cultures of bacteria incubated under conditions that promote nitrogen fixation, and in media with and without sources of haem. Assays will also include bacterial fractions isolated from active nodules. In addition, we will determine the extent to which leghaemoglobin recycling by plant cells contributes to CO production. These assays will be based on fractionation of nodules to produce bacteria-free preparations to which we will add various haem-containing proteins. Collectively, results from these studies and additional comparisons of activity under *in situ* conditions will establish the mechanisms by which legume nodules produce and emit CO.

9901051 Characterization and Functional Determination of Symbiosis-regulated Genes from an Ectomycorrhizal Fungus

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Grant 99 -35107-7810; $230,500; 3 Years

Ectomycorrhizal fungi are root-colonizing fungi that establish beneficial relationships with a wide variety of tree species. These fungi extend the absorptive capabilities of the root system, improve the host plant's abilities to tolerate biological and environmental stresses and support plant health under conditions of nutritional deficiency. Establishment of the mycorrhizal condition, with mycorrhizal fungi adapted to the existing environment, is essential to host plant health and survival. Thus, the mycorrhizal condition is an important factor controlling reforestation success, forest biomass production, and ecosystem health in general. However, little is known fundamentally about how the fungus and host plant establish the ectomycorrhizal relationship. Molecular genetic approaches have not been adequately exploited for study of the determinants of host specificity, recognition and symbiotic ability of these fungi. We have recently cloned symbiosis-regulated genes, PF6.2 and PFRAS, involved in initial recognition events from *Laccaria bicolor* that are expressed very early (6-24 h) during interaction with the tree host red pine. Thus, our objectives for this project are (1) develop transgenic *L. bicolor* strains with altered PF6.2 and PFRAS genes to determine the functional significance of these genes in the symbiosis process. This will be tested through the mycorrhizal ability of altered *L. bicolor* and (2) determine the temporal and spatial expression of symbiosis-regulated genes PF6.2 and PFRAS in mycorrhizal tissues. Through the proposed studies, we plan to unravel the molecular keys from ectomycorrhizal fungi that control the development of the symbiosis process. This information can be used to develop and utilize mycorrhizal fungi that are better equipped to support and improve tree health under various natural conditions resulting in increased forest biomass.

9900748 Biotic and Edaphic Controls of Ectomycorrhizal Community Structure and Function

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Grant 99-35107-7861; $329,500; 3 Years

Mycorrhizae are symbiotic associations between plant roots and fungi that assist plants to obtain nutrients. Ectomycorrhizae are important for forest tree growth. Many fungal species associate with few forest tree species, but the factors that control which fungi form mycorrhizae at any point in time or space are unclear.

This study will examine the role of soil heterogeneity in influencing the structure and function of ectomycorrhizal communities. Our three main objectives will be measure (1) the effects of nutrients or fungal antagonistic chemicals leaching from leaf litter on the competitive outcomes among ectomycorrhizal fungi; (2) the effect of grazing by soil fauna on competition between ectomycorrhizal fungi; and (3) the resultant outcome of competition in terms of nutrient uptake in manipulated ectomycorrhizal communities.

We will use pitch pine as a model host tree and manipulate the available leaf litter resource (single or mixed litters (oak, pine and blueberry) or their extracts), ectomycorrhizal community structure (by adding fungal inocula) and fungal feeding soil arthropod grazing density (by increasing to greater than field density). Experiments will be conducted in highly controlled microcosms, moderately controlled mesocosms and in the field (little control). These allow three levels of control of variation within experimental manipulations and allow interpolation of results from the laboratory to the field.

Greater understanding of ectomycorrhizal community structure and function will help forest management, selection of ectomycorrhizae for seedling tree inoculation prior to outplanting, and understanding the effects of pollutants and nutrient deposition which alter mycorrhizal communities.

9900759 Identification of Soil Physical and Hydraulic Properties Using Remote Sensing and Artificial Neural Network

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Grant 99-35107-7780; $107,000; 2 Years

The goal of this proposal is to examine the feasibility and robustness of estimating soil physical and hydraulic properties using *only* remote sensing observations. Multi-temporal soil brightness temperature and moisture maps will be used to physically relate soil moisture and soil physical properties. The proposed approach of identifying a distributed map of soil physical and hydraulic properties is based on recent developments in remote sensing of surface processes and Artificial Neural Networks. This methodology would allow estimation of soil physical properties with little or no information about site specific soil properties. Satellite and *in-situ* data from USDA experimental watershed in the Southern Great Plains will be used to test and validate the proposed methodology. Key objectives of this study are to: (1) identify soil physical and hydraulic properties using remote sensing observations and recent developments in artificial neural networks, and (2) examine the feasibility and robustness of the proposed approach by using data sets from several intensive field campaigns and *in-situ* measurements.

Expected results from this research will aid in developing spatially distributed models for flow and transport in the soil. Such models are invaluable for a wide range of water balance, irrigation scheduling, chemical transport, plant growth, and watershed studies. The proposed methodology of detecting physical linkages between remote sensing observations and soil physical and hydraulic properties expected from the Southern Great Plains has the potential to be translated to remote regions where very little or no site specific soil information is available.

9900859 Lignin Degradation in Woody Roots: Controls by Fungal Species, Nitrogen, and Manganese
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Grant 99-35107-7783; $318,000; 3 Years

Root decomposition is a fundamental but understudied process in forest ecosystems. The goal of our project is to understand how fungal species, nitrogen, and manganese control lignin degradation in Douglas-fir, western hemlock, ponderosa pine, and lodgepole pine woody roots in a series of chronosequence, laboratory incubation, and simulation modeling studies. First, lignin degradation in a chronosequence of root samples will be examined using nuclear magnetic resonance (^{13}C NMR) and near infrared reflectance spectroscopy (NIRS). These results will yield insights for developing a lignin/cellulose degradation model. Second, we will identify the fungal species involved in woody root lignin degradation using modern molecular methods. Third, we will examine the responses of lignin degradation in woody roots to nitrogen and manganese availability in laboratory incubation experiments. Fourth, we will improve an existing root decomposition model by adding the effects of white- and brown-rot functional groups on lignin degradation and overall woody root decomposition. Our proposed research will make a number of significant contributions to soil biology and ecological science that are important to sustainable agriculture. The presence of woody roots in soils directly influences soil strength. Our root decomposition data in the Pacific Northwest will be directly applicable to the question of how woody root strength changes after timber harvest, stand thinning, and forest fire. Our project is also relevant to the formation of "stable" soil organic matter and the carbon stores in forests after timber harvest, stand thinning, or natural disturbances such as fire.

9901056 Below Ground Changes in Ectomycorrhizal Fungal Diversity during Primary Succession
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Grant 99-35107-7843; $221,500; 3 Years

Under most conditions, greater than 95% of the short roots of an ectomycorrhizal (ECM) plant are colonized by fungi. These fungi replace root hairs, accessing soil nutrients and water for the plants. ECM fungi directly influence plant community dynamics, yet there are no below ground data that reveal which species are most important for plants establishing during primary succession in a natural habitat. We propose to address this utilizing a sand dune ecosystem undergoing primary succession. Shore pine (*Pinus contorta*) saplings are establishing in dune areas hundreds of meters away from contiguous forest stands. Some pines are establishing among vesicular-arbuscular mycorrhizal (VAM) grasses while others are establishing in the proximity of ECM shrubs. We will collect and identify ECM fruitbodies and use molecular methods to identify fungi directly from ECM root tips. We will test five hypotheses designed to: (1) compare the ECM fungal diversity on pines at various stages of primary succession to that of local contiguous forests, (2) identify important ECM fungi for pine establishment during primary succession, and (3) identify mechanisms that allow fungi from the local forest communities to establish with the isolated pines. The data will build on our understanding of ECM fungal succession and the functional roles of ECM fungi during plant establishment. From a management perspective, identifying species of ECM fungi tolerant to habitats such as the dune ecosystem will be important for dune stabilization with native plants specifically, and reforestation efforts in general.

9900862 Spatial Association between Root Length Density and Water Flow Paths in Highly Structured Soil
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Grant 99-35107-8268; $225,500; 3 Years

The information gained from this project will improve our ability to predict the fate of water, nutrients, and contaminants in soil. It will provide for a major conceptual breakthrough on the understanding of how plants influence the water cycle and water quality. The rationale for the research is that the availability of high quality water resources is a crucial issue for sustainable and enhanced land use. Nutrients and other water-borne contaminants escaping the root zone can adversely affect the quality of ground and surface waters. Plant roots influence the probability of nutrients and contaminants escaping to water supplies, but in clayey soils that occupy a large portion of land resources, the question of how plant roots influence the fate has not been answered. Plant roots grow in discrete locations in the soil, and water, nutrients, and contaminants flow in discrete locations in the soil. Information on how these two locations are spatially associated is critical in understanding water and nutrient cycles, but is not available. This lack of information hinders the proper design of long-term management practices aimed at protecting the quantity and quality of water sources. Our

hypotheses are that roots in clayey soil are associated with water, nutrient, and contaminant flow paths, and that this association influences the fate of water and chemicals. The objectives of the research are to determine the spatial associations between plant roots and water and chemical flow paths, and then to determine the significance of the associations to water management.

9900716 Electromagnetic Characterization of Soil Electrochemical and Geometrical Factors Affecting the Transport Process

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Strengthening Award; Grant 99-35107-7829; $277,000; 3 Years

Success of time domain reflectometry (TDR) prompted interest in other methods based on interations between electromagnetic (EM) fields and soil constituents. Moist soil constituents exhibit large changes in conductivity and dielectric constant at low frequencies (kHz to MHz) that reveal electrochemical properties and the spatial arrangement and interactions among various components. These low frequency responses have been used in geophysics to study brine saturated rocks and the resulting models may be modified and refined for soils. Non-invasive EM techniques will fill existing gaps in the *in-situ* identification of electrochemcial and geometric processes necessary for physically meaningful modeling of transport of nitrogen and pesticides through soils.

This project is a natural extension of an equipment grant awarded by NRICGP in FY 1998. Preliminary results investigating clay suspensions, show that meaningful and accurate measurements are made. Interactions of electric fields with ions electrostatically bonded to clay surfaces have been used to estimate diffusion coefficients of those ions and capture characteristics of particle geometry.

The research plan is in three phases that facilitates development of a physically based model. The first phase uses simple suspensions of reference clay and soil colloids where charge and particle size distributions can be carefully manipulated. The second phase uses cells packed with geothite-coated sand and sand-clay mixtures for extending model geometry from particle size to pore-wall size. In the second phase, the effects of variable saturation are added to the model. The final phase is testing and refining the model for non-destructive characterization of whole soils.

9901053 Linking Ammonia Oxidizer Communities to Nitrification Kinetics in Soils Treated with Dairy Wastes

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Grant 99-35107-7808; $252,000; 3 Years

Land–applied animal waste can be a major contributor to the component of water pollution resulting from agricultural activities. Nitrogen transformations in waste treated soils are of particular importance due to the potential for nitrogen in excess of plant demand to be transported off site causing surface and groundwater pollution. In soils receiving animal wastes, microbial mineralization, which transforms organic N to ammonium, and nitrification, which oxidizes this ammonium to nitrate, are crucial processes determining the availability and mobility of animal waste nitrogen. The factors controlling mineralization and nitrification in soils receiving different types of dairy waste (liquid, composted) will be assessed in this project. The bacteria responsible for the first step in nitrification, the ammonia oxidizing bacteria, will be examined using molecular techniques based on an essential enzyme. Changes in the types of ammonia oxidizing bacteria in response to waste applications will be followed over multiple years in silage corn. The field rates of mineralization and nitrification will be assessed concurrently. We believe that the ammonia oxidizing bacteria are a critical link in the efficient use of animal waste N to supply plant available N while preventing negative environmental impacts of excess NO_3^-. Our goal is to link the types of ammonia oxidizing bacteria, the supply of their substrate, ammonium, and the kinetics of the nitrification process into an improved understanding of the nitrogen cycle in soils receiving dairy wastes.

9900858 The Quantity and Form of Carbon in the Coarse Soil Fraction

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Grant 99-35107-7781; $132,500; 3 Years

Soil carbon is the second largest pool of carbon involved in global carbon cycling. Estimates of soil carbon have been used to develop models of global carbon cycling. These estimates of soil carbon are based only on carbon measured in the fine soil, not the larger, coarser soil materials. In addition, normally only soil to a depth of 1 m is considered, so estimates of soil carbon may be too low. As many soils are much deeper, much of the carbon stored in soils may not be considered. Likewise, recent work has shown that 15-20% of the carbon in a forest soil was bound to the coarse-sized soil particles. This research will measure total soil carbon in the coarse and fine soil fractions over a variety of soil types, and include the entire soil profile. In addition, we will analyze the organic coating found on the coarse particles to determine their composition and potential pathways of carbon in the soil. This data will give us a better understanding of carbon cycling in soils and a better estimate of global carbon storage. Likewise, it will give us better information about movement

and storage of carbon in different soils, including agricultural, forest, wetland and rangeland soils. Soil carbon is related to many other soil properties important to fertility, and understanding carbon in soils may yield important information about long-term productivity.

9900732 Verifying and Quantifying the Specific Complexation by Metals to Humic Substances

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Grant 99-35107-8072; $229,000; 3 Years

The objective of this project is to improve our understanding of trace metal chemistry in soil. Soil is made of both minerals and organic matter, the latter derived from decomposed organisms living in soil. Trace metals in soil bind to organic matter, a process that affects both their availability as nutrients or their potential toxicity to humans, animals or plants. Organic matter consists of complex molecules that have specific chemical structures to which the metal atoms bind. Subtle details in the manner trace metal atoms bind to organic matter has a profound influence on the solubility of these metals. For instance, it may be so weak as to render the trace metal readily available to organisms, or binding may be so strong that the metal atoms are unavailable. This study is a collection of experiments that examine whether minor constituents of organic matter (sulfur, nitrogen and phosphorus) play a far more important role in the binding of trace metals than previously thought. If, as we suspect, these minor constituents strongly affect trace metal binding, current predictions of trace metal fertility or toxicity are inaccurate.

9900736 Predicting Bioavailable Phosphorus Release from Animal Manures

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New Investigator Award; Grant 99-35107-8263; $96,000; 2 Years

Animal manures are applied to cropland based on rough estimates of nitrogen (N) availability over the growing season. Nitrogen-based application of manure often has led to soil phosphorus (P) overloading where P is susceptible to environmental loss. Concerns related to non-point nutrient pollution from croplands have generated state and federal initiatives to moderate P inputs to soils, including those from animal manures. Our ability to predict bioavailable P release from animal manures is currently hampered by a poor understanding of the factors controlling P mineralization and solubilization. The overall objective of this research is to develop a mechanistic understanding of phosphorus bioavailability from animal manures applied to soils. Specifically we propose to: (1) investigate the biological, chemical and physical factors controlling P release from dairy and poultry manure applied to a variety of soil types at different soil test P levels; (2) identify the P pools in manure that are most important to P mineralization and solubilization using physical fractionation, wet chemistry and spectroscopy (x-ray absorption, XAS, and near infrared) techniques and (3) evaluate the use of near infrared (NIR) spectroscopy as a tool for predicting P release from animal manures. This research should provide a mechanistic understanding of P availability from manures and improve our predictive capabilities.

WATER RESOURCES ASSESSMENT AND PROTECTION

Panel Manager - Dr. Lloyd Hossner, Texas A&M University
Program Director - Dr. Michael O'Neill

Land management and water use practices and policies affect the quality and availability of water resources. Research is needed on the effects of farming, range, forestry, and other agricultural practices on our water resources, and to develop effective and economically feasible water pollution prevention or remediation practices.

Innovative research is supported on: (a) the distribution, fate, and transport of water-borne contaminants of agricultural origin; (b) the role of soil heterogeneity, hydrology, and landscape position on water quality; (c) management and remediation practices and/or technologies; and (d) the social, economic, policy, and environmental impacts of agricultural land management and water contamination remediation practices.

9901008 *In Situ* Measurement of Water Content Using Gas-Phase Partitioning Tracers

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Grant 99-35102-8239; $270,500; 3 Years

Measuring the amount of water retained in soil (soil-water content) is important to many activities, including those associated with agriculture, forestry, hydrology, and engineering. For example, knowledge of soil-water content is especially important for management of agricultural resources, for flood control, and for predicting transport and fate of contaminants in the subsurface. The majority of the methods currently available for measuring soil-water content provide "point values" (i.e., they have small sample volumes). While this is an advantage for obtaining accurate information at small scales, it is a disadvantage for determining soil-water contents for larger (field) scales. Using current methods to determine the three-dimensional distribution of soil-water content at the field scale require what is typically a cost and time-prohibitive number of sampling locations. The overall goal of this project is to explore the use of an innovative *in situ* method for the larger-scale measurement of soil-water content in the vadose zone. The method is based on the use of a gas-phase tracer test, where wells are used to introduce a pulse of tracers into the subsurface. Selected tracers dissolve (partition) into the water, which retards their gas-phase transport relative to that of a conservative (non-partitioning) tracer. The retardation of the partitioning tracer is a function of the amount of water present. Thus, the results of the tracer test can be used to determine soil-water content. This project will evaluate the efficacy of the gas-phase partitioning tracer method, which if proven to be successful, will improve our ability to measure soil-water content in the subsurface.

9901109 Increasing the Sensitivity of Detection to Differentiate Animal and Human Wastes in Watersheds

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Grant 99-35102-8596; $118,000; 2 Years

The need for methods to specifically identify fecal contamination sources has increased as more emphasis is placed on reduction of pollutants at the source. The method we propose will allow differentiation among pig, cow, and human fecal pollution in water using specific toxin genes from *E. coli*. Calculations show that detection sensitivity needs to be increased by several orders of magnitude in order to effectively differentiate between these forms of fecal pollution. To increase sensitivity, we will use nested PCR, magnetic bead concentration of cells or DNA, and enrichment. We will use autoclaved stream water spiked with *E. coli* to test the sensitivity of each method. Upon completion of these experiments and processing of environmental samples, we expect to show that target toxin gene fragments are specific for cattle or swine fecal contamination in water. We also will know which concentration methods achieve the necessary sensitivity to make toxin biomarkers useful in identifying waste sources in water samples from agricultural watersheds.

9901032 Development of Novel OPH-based Materials for Detoxification of Organophosphate Pesticides

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Strengthening Award; Grant 99-35102-8597; $100,000; 2 Years

Synthetic organophosphorus compounds are used extensively as agricultural and domestic pesticides including insecticides, fungicides, and herbicides. These compounds are extremely toxic and act by inhibiting the nervous system of living organisms. Due to the growing environmental problems associated with these pesticides in food products and water supplies, there is a great need to develop safe, convenient, and economically-feasible methods for pesticide detoxification.

Enzymatic degradation of organophosphorus pesticides has been a subject of considerable attention during the past ten years. Organophosphorus hydrolase (OPH) isolated from soil microorganisms has been shown to degrade a wide range of organophosphate pesticides. However, the effectiveness of degradation varies among different pesticides, ranging from highly efficient with paraoxon to relatively slow with methyl parathion. This research will seek to "fine tune" and broaden

the substrate specificity of OPH *via* sequential cycles of DNA shuffling and screening. The ultimate goal is to build up a collection of novel OPHs that can be rationally combined to degrade a mixture of target pesticides.

9901134 Utilizing Seleniferous Agricultural Drainage Water in Production of an Animal Feed Supplement
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Grant 99-35102-8226; $152,000; 2 Years

The mineral selenium is essential for nutrition, yet toxic in large quantities. In the last 15 years, selenium has received much attention due to its accumulation and toxic effects in California's agriculturally important San Joaquin Valley. This natural source of pollution results from the region's high water table and intense irrigation, which leaches selenium out of agricultural lands into drainage water, posing a threat to wildlife.

Yeasts are known to accumulate selenate and selenite, the oxyanion forms of selenium often found in California drainage water. Yeasts are considered the rising stars in biotechnology. Uptake of selenium from contaminated drainage water by food-grade yeast will be assessed. The presence of selenomethionine in the yeast will be confirmed. Selenomethionine is found naturally in grains and is thought to have high bioavailability when fed to animals. The chosen strain of yeast will be used to seed a bioreactor which will be optimized for maximum selenium removal from drainage water so that environmentally safe levels are achieved and production of high yeast biomass is obtained. This novel approach to bioremediation will explore the bioaccumulation of selenium into yeast biomass with the resulting organoselenium available as a potential dietary supplement in animal feed.

9901101 Field Deployable Biosensors for Organophosphate Pesticides
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Strengthening Award; Grant 99-35102-8600; $250,000; 3 Years

At the time when the public concern about organophosphorus pesticide residues in food, water and the environment is increasing, the use of these pesticides in agriculture has also increased. New laws and regulations have been introduced and passed to ensure safe levels of these pesticides in our food and water supply. Sensitive, selective, rapid and reliable detection and measurement of organophosphorus compounds has become necessary for the protection of our food and water supply, the environment, and workers employed in agricultural and chemical manufacturing.

The goal of this collaborative research project is to develop, characterize and test novel enzyme- and microbial-based field deployable amperometric screen-printed biosensors for sensitive, selective, rapid, precise, accurate and inexpensive *in situ* testing of organophosphorus pesticides in ground and surface waters. The new amperometric enzyme strip electrode will rely on measuring the anodic current due to the electrochemical oxidation of phenolic compounds liberated during the organophosphorus hydrolase (OPH)-catalyzed hydrolysis of organophosphorus pesticides at an OPH-modified screen printed electrode. The project will thus couple the attractive biocatalytic activity of the OPH enzyme with the operation of low-cost, yet highly reproducible microfabricated strip electrodes. The thick-film (screen-printing) technology, has been widely used to fabricate enzyme biosensors used in home diagnostic applications for monitoring blood glucose, lactate and cholesterol. Indeed, most diabetes self-testing kits rely on screen-printing technology. It is expected that this multidisciplinary effort will lead to a commercial field-deployable/portable sensitive, selective, rapid, reliable, accurate and inexpensive biosensor for *in situ* monitoring of organophosphorus pesticides in ground and surface waters.

9901100 Innovative Technology Development for Prevention of Pathogen Migration Away from Feedlots
Grasso, D.; Smets, B.F.; Vinopal, R.T.
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Strengthening Award; Grant 99-35102-8593; $265,000; 3 Years

Migration of microbes such as *Escherichia coli* O156:H7 from feedlots may pose a significant public health risk. Dairy cattle have been identified as the primary source of *E. coli* O156:H7. Recently, this serotype infected a water supply thus causing human illness and death. This research will explore and develop a novel approach to mitigate this risk. Our approach is based on immobilizing bacteria by their interaction and subsequent adhesion to inorganic surfaces. We propose to use a three-step approach to identifying a soil mineral with beneficial adhesion capacity. First, we will determine surface interactions for different soil minerals and *E. coli* cells. Second, we will predict adhesion between the different soil minerals and the *E. coli*. This will assist us in determining whether or not adhesion is permanent or reversible. We will subsequently validate these results with experimental protocols. Finally, we will explore the impact of flow conditions through the use of column experiments. Based on our results an inorganic matrix will be suggested with appropriate adhesive properties for *E. coli*. The Abiobarrier© approach proposed here will yield a facile inexpensive technology that involves the use of mineral adsorbents that can be easily applied to feedlots to inhibit pathogen migration. This technology can assist farmers and

ranchers to insure that they properly protect the water supplies and consequently the public heath in an economical and reliable fashion.

9901040 Late-season Fertilization Effects on Turfgrass Growth and Nitrate Leaching
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Strengthening Award; Grant 99-35102-8598; $85,000; 2 Years

A significant portion of the managed landscape in Connecticut and other Eastern Seaboard states is covered with turfgrass. To maintain turfgrass vigor and quality, fertilizer is applied routinely. Late-season or "fall" fertilization of cool-season turfgrass is a well-established management practice throughout much of the northern United States and is usually beneficial for turf quality in most cases. Although late-season fertilization provides turf quality benefits, an appropriate cut-off date for the last fertilizer application has not been identified for most regions. Considering that climatic conditions during the fall period vary considerably from region to region, it can be reasoned that the last date of fertilizer application to turf should also vary accordingly. This, however, is not the case and fertilizer recommendations for many areas typically call for a November application or are vague as when to apply the last application, often just using the term "fall". Applying fertilizers beyond the date when cool-season turfgrasses are becoming dormant increases the potential of nitrate losses in runoff and leaching. The main goals of this study are to determine an appropriate cut-off date for fall fertilization of turf grown in southern New England that maintains acceptable turf quality while minimizing nitrate leaching losses. Fertilizers need to be suitably applied to turf because inappropriate or untimely applications may contribute to the nutrient enrichment of receiving surface and ground water.

9901157 Application of Phenotypic and Genotypic Methods for Detecting Fecal Pollution in Water Caused by Livestock
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Grant 99-35102-8595; $193,000; 2 Years

The bacterium *Escherichia coli* has been used as an indicator of fecal pollution of water for many years. However, in many cases it has not been possible to identify the source of the pollution. This is because *E. coli* is found in the intestinal tract of domestic and wild warm-blooded animals. Identifying the source of fecal pollution is important for evaluating human health risks and for implementing remedial actions. To date, there are no proven methods that accurately discriminate specific sources of livestock pollution from human and other domestic and wild animals. In this study, three methods will be used to characterize *E. coli* from human and livestock (swine, beef and dairy cattle). These methods are: analyzing for multiple antibiotic resistance (MAR); serological analysis; and genotypic characterization (ribotyping). It is anticipated that results of this study will be used to identify the sources of fecal bacteria in water.

9901133 Assessing the Vulnerability of Farmstead and Rural Domestic Wells to Agrichemical Contamination
Ray, C.
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New Investigator Award; Grant 99-35102-8551; $88,000; 2 Years

The goal of this project is to assess the vulnerability of farmstead and rural domestic wells to pesticide and nitrate contamination. Rural domestic wells do not come under any regulatory compliance for water quality testing. Periodic testing of individual wells is not feasible due to cost and logistics. However, the assessment of vulnerability of these wells, using data from some large-scale studies in the mid-continental United States is proposed. Artificial Neural Networks (NN) will be used for vulnerability assessment utilizing the occurrence data of nitrate and pesticides, well site land use information, well construction details, and other climatic, hydrologic and geologic data. The NNs are multi-input/multi-output models that have the capability to account for complex interaction among the input parameters that contribute to domestic well contamination. The NNs have been successfully used in other hydrologic applications such as rainfall-runoff modeling. As more pesticide and nitrate occurrence data become available along with site information, the NN model can be retrained with the new data and the model can be tested against sites with such data. Using a trained and tested NN model, contamination potential for new sites with adequate site and hydrologic data can be predicted in the same region. The identified high-risk sites can be taken up for confirmatory sampling by the health or regulating agencies. The contaminated wells thus can be identified for remedial measures and the well user can take other measures to prevent future contamination. Potential cost savings can be realized by testing only the most vulnerable wells.

9901010 Closed-Loop Precision Irrigation for Improved Water Management

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Strengthening Award; Grant 99-35102-8575; $98,000; 2 Years

One of the technological barriers to commercialization of precision irrigation is the lack of a cost effective means for determining spatially variable irrigation requirements. This project targets the development, testing, and evaluation of a closed-loop decision support system to effectively determine spatially variable irrigation requirements. The system consists of spatially distributed micro-instrumentation units linked to a center pivot supervisory control and data acquisition network using a low-power radio frequency link. The micro-instrumentation units will be equipped with sensors to monitor soil water content, water application, and environmental parameters within and above the crop canopy. Data collected by the micro-instrumentation units will be maintained at the center pivot control point for retrieval by a portable computer. A simple decision support model for determining spatially variable irrigation requirements will be developed. The micro-instrumentation units linked to the center pivot control and data acquisition network will provide near real-time information to drive the decision support system. The closed-loop control system will be developed and tested using data from artificially induced spatially variable irrigation requirements under controlled field conditions. Performance of the control system will be evaluated based on ability to maintain target soil moisture levels under induced variability and resulting crop yield and quality.

9901007 Water Quality Protection using Novel *Brassicaceae* Cover Crops

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Grant 99-35102-8237; $165,500; 2 Years

Protection of water quality from possible contamination by agricultural crop production practices requires integrated approaches at the field level. Our emphasis is on using mustard and rapeseed cover crops to ensure water quality by reducing pesticide contamination, decreasing erosional losses, and increasing nutrient use efficiency. More specifically, we propose that novel *Brassicaceae* varieties developed at the University of Idaho (1) contain natural allelochemicals that are pesticidal toward plant pests called nematodes, (2) decrease erosion through substantial residue production, and (3) reduce the potential for nitrate leaching into groundwater. The substitution of plant-derived allelochemicals for synthetic pesticides decreases the possibility of environmental contamination, thereby reducing associated negative impacts on human health. Likewise, reduced erosion and nutrient leaching serve to decrease negative impacts on water quality. We will conduct field studies to determine if these novel cover crops provide water quality benefits, while maintaining or enhancing economic returns in potato production systems. We will plant seed from new species produced by crossing rapeseed and mustard into field plots established at the Washington State University Irrigated Agriculture Research and Extension Center, located in Prosser, Washington. Cover crops will be planted in the fall and incorporated into soil in the spring before planting potatoes, thereby releasing the natural pesticides. Benefits to potato production and environmental quality will be assessed during each of two years. By project completion we will thus have assessed the potential water quality benefits of including these unique cover crops into an established cropping strategy.

9901086 Alliance for Environmental Stewardship: A Comprehensive Approach

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Grant 99-35102-8236; $8,000; 1 Year

Nitrogen and phosphorus are nutrients essential for plant and animal life that become pollutants when concentrated. Nutrient pollution is responsible for eutrophication of lakes and bays and high nitrate levels in drinking water associated with blue-baby syndrome. Nutrients released into the environment are produced by (1) animal agriculture, (2) commercial fertilizer, (3) biosolid residuals (sludge) from human and animal waste treatment facilities, (4) urban components, and (5) on-site wastewater/sewage treatment (septic tanks). Sixty-one speakers and 125 invited key personnel that equally represent each of these sources, and the public, will have a comprehensive, objective, and science-based discussion of the impact on our environment by nutrients at a meeting titled, “Alliance for Environmental Stewardship: A Comprehensive Approach,” on September 27-29, 1999, in St. Louis, MO. Workshop goals include: (1) provide an opportunity to cooperate with representatives for all sources of nutrients, and persons who influence policy (e.g., media) to discuss ways in which we can work together to protect the environment; (2) create recognition that all sources of nutrients impact the environment; (3) promote sound decision making processes that require scientific and comprehensive evaluation; (4) define and implement proactive programs through commercial agencies, government agencies and universities; and (5) develop a set of prioritized recommendations that decision makers may use as a basis for comprehensive policy development, and establishment of research and economic priorities that will correct or ameliorate pollution problems. Papers, poster abstracts, and prioritized recommendations published in the proceedings, will be available to the public.

9901108 Innovative N Management Systems for Corn: Source Reduction to the Gulf of Mexico
Scharf, P.C.; Blackmer, A.M.; Kitchen, N.R.; Davis, J.G.; Lory, J.A.; Sudduth, K.A.
University of Missouri; Department of Agronomy; Columbia, MO 65211
Grant 99-35102-8594; $223,000; 2 Years

Each summer a large low-oxygen zone forms on the Louisiana coastal shelf in the Gulf of Mexico, driving away fish and killing bottom-dwelling animals. The formation of this zone is fueled by nitrogen from the Mississippi River, which has increased dramatically since 1950, mainly from agricultural sources. Nitrogen fertilizer is often over-applied to crops to make sure that they have enough. Few tools have been available to farmers to match nitrogen fertilizer rates to actual crop needs. We will be conducting field-scale tests of four promising N recommendation tools that we have developed to see if they can maintain profitability while reducing over-application of nitrogen to corn.

9901020 The Role of Hyperfiltration in Fate and Transport of Fertilizers in Shallow Perched Aquifers
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Strengthening Award; Grant 99-35102-8524; $135,000; 3 Years

Shallow perched aquifers are common in agricultural areas and are subject to contamination from many sources including fertilizer application and nitrate from feedlots and dairies. Presently, there is no method for predicting water and solute volumes that pass through aquitards underlying perched aquifers, nor is there a method available to predict how much solute is retained in the perched aquifer. Darcy's Law does not apply because the shales and clays that form the aquitards can function as non-ideal membranes and retard the passage of solute. This study will perform experiments that focus on the passage of the dissolved fertilizer components nitrate and phosphate through clay layers. If clay membranes can concentrate nitrate and phosphate, as theory strongly suggests, microbial activity may be favored near aquitards. Therefore, the experiments will be performed abiotically at heads representative of perched aquifers. Various models, including both steady state and transient approaches, based on the non-equilibrium thermodynamics of membrane processes, will be further developed and tested against the experimental data. The project's deliverables will be (1) an extensive experimental data set for low-head clay membrane experiments against which mathematical and numerical models can be tested, (2) a practical model for calculating membrane effects during groundwater flow through clay layers, and (3) a quantitative basis for further investigation of the relationship between clay membrane processes and subsurface microbial metabolism. The knowledge gained by this study is of prime importance in furthering understanding of the fate and transport of fertilizer components in the subsurface.

9901095 Anodic Fenton Treatment and Toxicity Evaluation of Aqueous Pesticide Wastes
Lemley, A.T.; Bloom, S.E.
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Grant 99-35102-8238; $230,000; 3 Years

The overall goal of the project is to optimize a new treatment method developed by the principal investigators for pesticide wastes. It has been established that pesticide rinsate and other pesticide wastes are a significant environmental hazard for water supplies. The project represents a major contribution to environmental protection by agriculture. The new method makes use of a simple electrochemical cell through which large volumes of pesticide rinsewater or wastewater can be processed and made less toxic. The investigators have tested this new method with a small sample of pesticides under laboratory conditions. The simple lab model must be modified with appropriate technology, including membranes to separate the electrochemical half cells, so that it can be used as a flow through system in the field. Future work will involve studies of a wider variety of pesticides, mixtures of pesticides, and actual tank mixtures used in agriculture. It will be critical to analyze the treated wastewater to determine the chemical nature of the degradation products and to test them for toxicity. These toxicity tests, which include assessment of toxicity at the molecular and cellular level, can show that the treated wastewater is considerably lower in toxicity than the untreated wastewater.

9901125 Hydrologic Control of Nitrogen Transport at Hydrogeomorphic Interfaces in a Forested Watershed
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Strengthening Award; Grant 99-35102-8588; $100,000; 2 Years

Recent evidence indicates the importance of hydrogeomorphic interfaces (e.g., bedrock/till, till/peat, peat/alluvium, alluvium/channel) in controlling temporal N transport in streams. Underlying glacial deposits and deep groundwater reservoirs may be a major contributor to N pulses seen in many eastern streams, but locating and sampling them are problematic in these glacially-mantled watersheds. We are using spatial and temporal sampling strategies to characterize the contribution of these deep sources of N to a stream channel on a continuum from hillslope, wetland, hyporheic and littoral zone waters in an Adirondack watershed in New York. Ground penetrating radar (GPR) will assist us in locating the

depth of deep groundwater sources, in addition to the vertical heterogeneity in hydraulic conductivity in wetland and hyporheic deposits. Measurements of water table fluctuations within wetlands intersecting primary drainage channels (hydroperiod determination), along with tracing of preferential flow controlled by alternating layers of peat and alluvium, will assist in the determination of the spatial and temporal contribution of deep N sources to streamwater N-concentrations. In addition, subchannel sampling of hyporheic inputs (mini-piezometers) will allow us to interpret the seasonal and episodic contribution of hyporheic deposits to N-signature. Results will be generalized to the region through ground-truthing at other wetlands within the Upper Hudson River basin. This information will be coupled to a digital elevation model (DEM) of the watershed, and long-term monitoring of overall N flux from the watershed, to develop a better understanding of N pollution inputs, and to determine the most critical hydrogeomorphic interfaces controlling N transport.

9901013 Effect of Subsurface Drain Depths on Nitrogen Losses to Surface Waters

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Grant 99-35102-8571; $300,500; 3 Years

Effects of nitrogen (N) losses to surface waters are of great concern on both national and regional scales. There is increasing evidence that large areas of hypoxia in the Gulf of Mexico and related water quality problems in estuaries along the Atlantic Coast are due to excessive nitrogen derived primarily from agricultural sources. Some of the highest losses of N to surface waters comes from lands with subsurface drainage. These lands are among the world's most productive soils, which make up an extremely important part of our cropland base. There is a clear need to develop methods for drainage and continued agricultural production on poorly drained soils that will reduce N losses to surface waters. Computer modeling studies and limited field studies indicate that N losses can be significantly reduced by using relatively shallow subsurface drains. However, there is not sufficient experimental evidence at this time to either quantify the effects of drain depth on N losses or to serve as a basis for recommending the practice.

We propose to conduct field scale studies to investigate the effects of drain depth on N losses from artificially drained lands. A three-year field experiment will be conducted to determine the effects of drain depth on nitrogen losses from drained agricultural cropland.

The research will be conducted on a North Carolina Coastal Plain site to compare drainage outflow and N losses from drains at depths of 75, 115, and 150 cm. In each case, the drain spacing will be selected to satisfy agricultural production requirements. The experiments will also determine if the use of shallow drains to reduce N losses will affect losses of sediment and phosphorus (P) *via* surface drainage. The results will be used to test and further develop DRAINMOD based simulation models for describing effects of drainage system design and management on the water balance, crop yields and N, P, and sediment losses in terms of soil, site and climatological variables.

9901026 Evaluating Management Practices to Reduce Microbial Impacts of Animal Wastes on Water Quality

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Grant 99-35102-8178; $185,500; 2 Years

The management of manure (feces, urine and other animal wastes) from animal feeding operations (AFOs), especially the high-density or concentrated ones, is of growing environmental and public health concern because of the increased growth and density of this form of animal agriculture. The environmental and public health concerns include the possible disease-causing microbes in animal fecal wastes and their potential to contaminate water resources used for beneficial purposes, such as drinking water sources, primary contact recreation (swimming), fruit/vegetable irrigation and raising edible shellfish. The objectives of this study are to determine: (1) the types and amounts of human pathogenic microbes in untreated AFO manures from swine and cattle and (2) the byproducts of these microbes and the fecal indicators for them in alternative waste treatment systems (lagoons, constructed wetlands and other biological processes) and related management practices (land application/spreading, waste solids drying, etc.). A third objective is to determine the extent to which current and candidate best management practices (BMPs) for AFO manures result in microbial contamination of adjacent and nearby surface waters. Results will be determined seasonally and as a consequence of specific events, such as land application of the wastes and rainfall events. This investigation of microbes in raw and treated AFO animal wastes and potentially impacted surface waters will use advanced, state-of-the-science "molecular fingerprinting" and other analytical techniques to detect, quantify and characterize the microbes in order to conclusively determine if concentrated AFO manures are important sources of contamination of surface waters with bacterial, viral and parasitic pathogens that infect humans. The relationships or predictive associations, if any, between microbes and other water quality parameters also will be determined.

9901119 Subsurface Riparian N Removal: The Role of Landscape Setting

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Grant 99-35102-8266; $285,000; 3 Years

Reducing nitrogen inputs to coastal waters is a major challenge for American agriculture. Riparian buffer zones (vegetated lands that border streams) hold great promise for removing nitrate-nitrogen that escapes from agricultural activities. However, riparian buffers exhibit a large degree of variation in their capacity for nitrate removal. There is great concern that riparian zones are being applied as tools for nitrate removal in many areas where they may not be effective. In this project we will explore the interactions between site characteristics and the nitrate removal capacity of riparian zones. We will then use our results to identify specific ecosystems and landscapes where riparian zones are likely to be effective tools for groundwater N removal. Our objectives are to: (1) quantify rates and extent of groundwater NO_3^- removal in soils of different drainage class on riparian forests developed on glacial till and glacial outwash and 2) conduct detailed analysis of shallow aquifer physical, chemical and biological properties, and how these properties vary with water table dynamics in the subsurface of riparian forests on glacial till and glacial outwash**.** We will measure *in situ* groundwater nitrate removal in a range of sites to determine the site factors controlling groundwater NO_3^- dynamics in different geomorphic settings. We will couple those results with estimates of groundwater flowpaths to evaluate the role of different hydrogeomorphic settings on riparian zone watershed N dynamics.

9901027 Quantifying and Minimizing 17 -estradiol Discharges from Dairy and Swine Waste

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Grant 99-35102-8179; $215,500; 2 Years

Hormonal pollution of the environment by compounds such as 17 -estradiol has been implicated in human and wildlife health problems. However, little is known regarding the environmental impact of hormones from farm animals. Such knowledge is timely, since current animal waste regulations are driven solely by concerns about nutrients, pathogens, and organic matter; no consideration is given to the impact of these regulations on environmental estrogen loads. Previous work examining hormone discharges from livestock have focused on poultry waste; little has been reported regarding the potentially large environmental loadings of estrogen from dairy and swine operations. To address this gap in knowledge, this work has four objectives. First, measure 17 -estradiol inputs and outputs in dairy and swine waste storage systems, to see whether estrogen degradation is significantly different between systems. Second, measure 17 -estradiol concentrations in runoff from plots fertilized with liquid swine waste and solid dairy wastes, to document the potential environmental significance (or insignificance) of these losses. Third, determine the efficacy of high-rate anaerobic digestion for degrading 17 -estradiol. Fourth, determine how 17 -estradiol is degraded in dry stacks, lagoons, high-rate anaerobic digesters, and soil. This will be done in laboratory studies that will determine how microbes from dry stacks, lagoons, high-rate anaerobic digesters, and soil, degrade 17 -estradiol.

9901097 Predicting Offsite Subsurface Migration of Agrochemicals-Noninvasive Surveying

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Grant 99-35102-8273; $245,000; 3 Years

Offsite movement of waterborne agrochemicals is increasingly targeted as a nonpoint source of water quality degradation. Our research has shown that subsurface water movement is highly variable and site-specific, and that a small soil volume often conducts a large volume of water flow. Concentrated flow in small soil volumes is usually caused by soil structural features and causes subsurface water flow to move rapidly offsite from certain areas of fields, while little or no lateral subsurface flow occurs in other areas. An accepted conservation practice to reduce surface erosion is to employ special management for high-risk areas. Similarly, it may be necessary to alter management practices within production areas with a high potential for offsite subsurface water flow. It is difficult to identify these subsurface regions using conventional soil survey and vadose zone sampling techniques. Our preliminary research on loessial soils in the Southern Mississippi Valley Silty Uplands suggests that it may be possible to locate areas of rapid subsurface offsite water movement by combining electromagnetic induction (EMI) and ground-penetrating radar (GPR) surveying techniques. By developing a protocol that employs traditional surveying, EMI, GPR, and geostatistical modeling to identify areas that are susceptible to subsurface offsite movement of agrochemicals and by optimizing the identification techniques under different soil water contents and at field-scale, this research will provide agricultural producers with a tool to identify areas of rapid subsurface flow of water and agrochemicals. Identification of these areas will provide the producer an opportunity to employ best management practices to prevent the offsite movement of agrochemicals.

9901014 Pesticide Fate and Removal in Constructed Wetlands

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Strengthening Award; Grant 99-35102-8523; $110,000; 2 Years

Growing nursery stock in containers under highly controlled conditions has been a practice increasing in popularity in the United States. An environmental consequence of containerized plant production is the fertilizer and pesticide-laden runoff generated by frequent and intense irrigation. Seventy to eighty percent of the water applied to a container crop by overhead irrigation falls between the pots or in the aisles and is lost to runoff. Research shows that bedcover composition is a significant factor in herbicide movement after application and irrigation, and runoff quantity from nurseries has increased with synthetic (fabric or plastic) or packed clay groundcovers.

Herbicides are applied in granules or by broadcast spraying, which is the most popular method. With containers spaced 30 cm apart, as much as 80 percent of broadcast applications fall on the bed cover instead of containers. Frequently, herbicides are sprayed directly on the groundcover to prevent weed growth, and with overhead irrigation typically following herbicide application, the granules or spray residues are carried with the runoff water. Aquatic organisms, wildlife, non-targeted vegetation, and humans can potentially be negatively impacted by contact or ingestion of these herbicides.

Research conducted by Tennessee Technological University has shown that subsurface constructed wetland systems can potentially remove herbicides in nursery runoff. Based on previous research, the proposed study will determine the herbicide removal processes and develop design equations or guidelines.

9901033 Improving Nitrogen Fertilizer Use-Efficiency in Deficit-Irrigation Systems for Cotton in the Southern High Plains

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Grant 99-35102-8526; $318,500; 3 Years

Cotton production on the nearly 1.1 million ha of the Texas Southern High Plains is limited first by lack of water and, second, by lack of nitrogen (N). Irrigation water, delivered mostly by center-pivot, comes from the declining Ogallala aquifer and is applied to about half of the cotton in the region. The development and wide adoption of the highly efficient Low Energy Precision Application (LEPA)-irrigation system, which uses low-pressure, frequent applications close to the soil surface, has allowed irrigated cotton farming to continue in this semi-arid region of high winds and low rainfall. Nitrogen management under LEPA-irrigation and the small but growing sub-surface-irrigation (SDI) system has not received nearly as much attention as has the water management. Irrigated cotton farmers are gradually increasing their N application rates, resulting in increasing lint yields, however, the groundwater NO_3^- levels are also increasing. Our hypothesis is that irrigation management systems that are more efficient in terms of water-use are also more efficient in fertilizer N-use and in minimizing NO_3^- leaching. We also hypothesize that optimal N management for cotton will differ according to irrigation system and soil type. In the multi-disciplinary structure for research in agriculture called CIAPSE (Center to Improve Agriculture Productivity in Semi-arid Environments) we propose detailed study of the fate of ^{15}N-labeled fertilizer in SDI, LEPA-, and spray-irrigation on two representative soil types planted to cotton. The objectives of the research are to: (1) compare N fertilizer use-efficiency by cotton and NO_3^- leaching among three different irrigation systems, and (2) develop N management decision aids for cotton in the three irrigation practices for varying initial soil NO_3^- scenarios and fertilizer to cotton lint price ratios. The information generated on N cycling and N management in the reduced-water irrigation systems will have the impact of more profitable cotton production and protection of groundwater quality in the Southern High Plains.

9901085 Quantifying the Exposure of Streams to Sediment Inputs from Managed Forests, a Risk-based Approach

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Strengthening Award; Grant 99-35102-8599; $279,500; 3 Years

This project will develop and test a risk-based management tool for quantifying the potential frequency and magnitude of sediment input to streams in managed forests. This work will emphasize the characterization and integration of the frequency-magnitude character of processes that combine to contribute sediment to stream channel networks. A key objective is to adapt existing deterministic basin-scale upland sediment models for use in a comprehensive risk-based framework. Formal risk analysis techniques are highly developed in other disciplines and provide an ability to take into account uncertainty in model parameters, and characterize the variability inherent in the processes being modeled. Chains of events including climatic triggers that lead to landslide and erosion events that in-turn lead to sediment transport and input to streams will be characterized across the landscape. The effects of managed forests in modifying the governing variables and altering the frequency magnitude curves of sediment input will be estimated for use in making forest management

decisions. The testing of this approach will be conducted within an area that has been extensively documented as having severe sediment impacts on streams in central Idaho. This work will directly address the need for research to increase our understanding of forest ecosystems and management practices to optimize our stewardship of forest ecosystems. It also addresses the need to reduce the release of waterborne contaminants of agricultural origin.

NUTRITION, FOOD SAFETY, AND HEALTH

The health of the U.S. citizen depends on the quality and quantity of the country's food supply and the nutrients consumed by individuals. Research is supported which will contribute to the improvement of human nutritional status by increasing our understanding of requirements of nutrients and factors affecting optimal human nutrition. Grants are also awarded in the area of food safety, particularly emphasizing the detection, prevention and control of food-borne disease-causing microorganisms and naturally occurring toxicants.

IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH

Panel Manager -Dr. Kenneth G. D. Allen, Colorado State University
Program Director - Dr. Debora Hamernik

This program area emphasizes research that contributes to our understanding of appropriate dietary practices throughout the life cycle and factors that affect these requirements such as gender, race and ethnicity. In addition, new insights are needed about factors that affect the attitudes and behavior of consumers toward food. The following areas of research will be emphasized: nutritional requirements including metabolism and utilization for all age groups, bioavailability of dietary components, the interrelationships among dietary components, mechanisms underlying the relationship between diet and optimal health (such as the effect of nutrients on the immune system) and cellular and molecular mechanisms influencing nutritional status (including nutrient effects on gene expression). In addition, this program supports research on identification of obstacles to adopting healthful food habits with particular emphasis on factors affecting consumer attitudes and behavior and development of recommendations for interventions to improve nutritional status. Innovative approaches to these research problems are encouraged.

9900771 Modulation of Apolipoprotein A-I Gene Expression by Zinc Status
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Grant 99-35200-7620; $250,000; 3 Years

Apolipoprotein (apo) A-I is the principal protein of high-density lipoprotein (HDL). HDL is produced in the liver and intestine for the removal of cholesterol from peripheral tissues. Low plasma HDL and apo A-I levels are associated with an increased risk of cardiovascular disease (CVD). In the United States, marginal dietary zinc (Zn) deficiency may be a common problem and may contribute to the development of CVD because of the reduction in HDL and apo A-I observed in Zn-deficient animals. In this project, a human liver derived cell line, Hep G2, will be cultured in deficient and adequate levels of Zn and used to examine the role of Zn in the regulation of apo A-I gene transcription. This process involves the binding of nuclear factors to specific elements within the gene's promoter region, which turns on the duplication of the downstream DNA sequence in the formation of messenger (m) RNA. Cell-free transcription studies, using isolated cell nuclei, will be used to establish whether a decrease in transcription rate of apo A-I gene may contribute to altered level of cellular mRNA. The apo A-I gene promoter activity will be measured by transfection of apo A-I reporter genes into Hep G2 cells. In addition, the contribution of metal responsive elements (MRE), within the apo A-I promoter, to the modulation of promoter activity will be examined with mutated apo A-I reporter gene constructs. The influence of Zn status on specific interaction of MRE with nuclear factors will be examined and these nuclear factors will be isolated and characterized.

9900772 Fourth International Conference on Dietary Assessment Methods
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Grant 99-35200-7585; $5,000; 1 Year

The current conference proposal is to support the initial costs of announcements and mailings for the Fourth International Conference on Dietary Assessment Methods. The Conference will be held in Tucson, Arizona from September 17 to September 20, 2000. There is a local organizing committee and an international advisory committee working to 1) develop sessions, 2) identify speakers, 3) participate in sessions, 4) review abstracts, 5) assist with the conference promotion, and 6) obtain financial support. We anticipate an attendance of 500 scientists from about 30 countries. The conference will address several of the "Healthy People 2000" priority areas such as 1) nutrition, 2) maternal and infant health, 3) heart disease and stroke, 4) cancer, and 5) diabetes and chronic disabling conditions. Dietary methods are the cornerstone for conducting epidemiological and intervention studies on diet and health. This conference will have plenary and breakout sessions dedicated to methods for determining the extent of food insecurity and hunger in developed and developing countries, and sessions on measuring the dietary intake of nutrients and non-nutrients that are associated with health. There will be presentations on improved data collection methods and statistical methods for improving analysis of data. Specific conference aims are the following: 1) Provide a forum for sharing new knowledge and ideas; 2) Promote a better understanding of current dietary assessment methodologies; 3) Stimulate interdisciplinary approaches to research; 4) Focus attention on methodological issues; 5) Address methodologic issues of international studies; 6) Identify progress and future research priorities; and 7) Publish the proceedings.

9900684 Plant-Based Food Intake and Cardiovascular Disease Risk With a Focus on Ethnicity and Gender
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Postdoctoral Fellowship; Grant 99-35200-7605; $89,982; 2 Years

National nutrition education campaigns emphasize a restriction of fat and cholesterol. Unfortunately, the low-fat, low-cholesterol (LFLC) educational method does not ensure a heart-healthy diet due to widely available processed low-fat low-cholesterol foods that are low in fiber and nutrients and high in sugars. Sugar-rich, low-fiber diets are commonly lacking in important nutrients and phytochemicals which are associated with a favorable cardiovascular disease (CVD) risk status. Little information is available on plant-based food intake (PBFI) in the US in relation to CVD risk factors, particularly among selected ethnic groups. Therefore, the applicant will study the role of PBFI in relation to CVD risk factors in over 20,000 African-American, Mexican-American, and White men and women using data from the Third National Health and Nutrition Examination Survey (1988-1994). It is believed that higher levels of PBFI will be associated with a favorable CVD risk status, independent of dietary saturated fat and cholesterol. CVD risk status will be based on several factors including blood cholesterol, blood pressure, obesity, diabetes, and blood nutrient levels. This project will provide valuable knowledge on what patterns of PBFI are associated with low CVD risk among selected ethnic groups and varying socioeconomic levels. Additionally, this knowledge will allow for the development of culturally specific educational tools to promote PBFI in accordance with USDA nutrition objectives which include increasing dietary intakes of fruits, vegetables, whole grains, and beans.

9900651 Effect of Fish Oil on Hepatic Lipid and Carbohydrate Metabolism in Healthy Human Subjects
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New Investigator Award; Grant 99-35200-8605; $150,000; 3 Years

Large-scale studies have confirmed that the frequency of cardiovascular disease was much lower in Eskimos who consumed traditional foods when compared to populations consuming Western diets. This difference was not explained by the quantity of dietary fat (similar in the Eskimo and Westerners) but by the source of fat (fish versus land animal and plant, respectively). These observations sparked extensive investigations, which determined that the beneficial effects could be attributed to fish oil. It is now recognized that fish oil has a wide range of biological effects on blood fat, platelets, blood clotting, blood pressure and others, with the potential to protect against the risk of cardiovascular disease. More specifically, blood levels of a particular fat called triglyceride, which is associated with cardiovascular disease, can be reduced by 20 to 40 percent. At present, although fish oil has a recognized effect on lowering triglyceride levels, it is not known how this effect operates. The consequences of this effect on liver glucose metabolism are also unknown. We propose that: 1) the reduction of triglyceride by fish oil is due to a reduction of the conversion of carbohydrate to fat in the liver, and 2) fish oil may increase glucose synthesis and production by the liver. To find out whether this proposal is true, we will measure the synthesis of fat and glucose in healthy subjects when they are receiving either fish oil or a placebo in their diet. This study will provide important information for understanding the mechanism of fish oil actions.

9900815 Metabolic Genomics: A Novel Technology to Assess Nutrient Status
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New Investigator Award; Grant 99-35200-7578; $230,000; 2 Years

With every meal the body extracts from the diet the nutrients required for its needs. For every nutrient from the abundant, such as proteins and carbohydrates, to the scarce, including vitamins and minerals, complex mechanisms regulate uptake from diet, utilization by the body, and excretion of excess. Disease, inadequate diet or excessive nutrient loss can disrupt this delicate balance and detrimental nutrient deficiency or excess results. Clinical assessment of nutrient status of an individual is complicated by the vast number of required nutrients, the intricate mechanisms for balancing need and excess, and the subtle clinical findings of nutrient deficiency or excess. In the absence of characteristic findings present in severe nutritional disorders, a clinician is faced with a bewildering selection of possible diagnostic tests for a suspected nutritional disorder. Alternative robust methods able to simultaneously assess multiple nutrients, tolerant of the complex interactions between nutrients and yet able to discern specific nutritional disorders, are needed. We propose to develop a novel methodology to assess the nutritional status of an individual by monitoring the metabolic response of the individual to changes in its environment which may provide the means to determine the nutritional state of an individual. We will monitor one critical aspect, changes in gene expression, as an indicator of the metabolic response to nutrient deficiency or excess. Recognizable nutritional response patterns could then be used in clinical assessment to monitor the nutritional status of an individual or to identify a nutritional deficiency or excess.

9900780 Absorbed beta-Carotene: A Retinoid Source in Humans

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Grant 99-35200-7584; $175,000; 2 Years

Dietary beta-carotene is absorbed from the intestine as a mix of intact beta-carotene and its derivatives that include vitamin A and other retinoids. Therefore, the role of beta-carotene that is absorbed intact in health remains controversial for lack of basic information about its post-absorptive metabolism. Limited evidence demonstrates that many tissues and organs metabolize beta-carotene to vitamin A active compounds like retinoic acids that are essential in maintaining cell function, but this event has yet to be demonstrated in humans. Therefore, quantifying the degree to which beta-carotene that is absorbed intact serves as a source of retinoids has emerged as a high priority. We will determine the metabolic fate of a minute ($1x10^{-15}$ g) and ethically acceptable amount of ^{14}C-beta-carotene in healthy humans. We will use Accelerator Mass Spectrometry because it can detect these small amounts of ^{14}C-labeled compounds. A small blood sample (a teaspoon) will be drawn and labeled in a tube with a minute amount of ^{14}C-beta-carotene and returned intravenously to its donor, thus circumventing intestinal absorption. Time-course kinetics of this labeled beta-carotene and its derivatives will be determined over a 60-d period in plasma, urine, and feces. The results will elucidate pathways by which beta-carotene is converted to vitamin A and other retinoids. It will suggest dietary intervention and therapeutic strategies for optimal health. This proposal is central to the mission of the NRI because it will characterize inter-conversions among food components and elucidate their individual contribution to optimal health of Americans: the stakeholders.

9900698 Metabolic Effects of a High Fructose Diet in Women: Role of Leptin

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Grant 99-35200-7763; $250,000; 2 Years

Obesity is a major risk factor for type 2 diabetes. Obesity is also associated with increased blood pressure (hypertension) and increased levels of cholesterol, both of which are linked to cardiovascular disease. The prevalence of obesity in the U.S. has also increased. Fructose does not stimulate the release of the hormones insulin and leptin into the bloodstream, whereas another dietary sugar, glucose, stimulates both insulin and leptin production. Insulin and leptin are two important signals to the brain which regulate appetite and metabolic rate. Since fructose consumption does not activate these long-term signals of body fat content and energy (calorie) intake, the brain in effect does not count calories consumed as fructose. Therefore, the appropriate adjustments of appetite and metabolic rate are not made to compensate when excess energy is consumed as fructose. This suggests that diets high in fructose would contribute to weight gain and obesity, and therefore to increased prevalence of type 2 diabetes. To experimentally test this idea, we will compare the effects of diets high in fructose with those high in glucose on insulin and leptin levels, appetite, weight gain, body fat content, and metabolic rate in moderately overweight post-menopausal women before and during an 8-week test period. Since a decrease in the effectiveness of insulin to regulate blood sugar is observed in obesity and type 2 diabetes, as well as elevations of blood pressure and cholesterol levels, these will also be measured.

9900663 Low Phytate Corn Mutants

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Grant 99-35200-7603; $275,000; 2 Years

Phytic acid is a substance that is present in virtually all plant foods, especially grains and legumes. Phytate is considered to be the principal factor in those foods that impairs the absorption and bioavailability of such nutritionally important minerals as zinc and calcium. Low phytate corn has been produced at the USDA-ARS National Small Grain Germplasm Research Facility using simple mutation genetics. Because phosphorous is better absorbed from this grain, thus reducing phosphorous pollution from livestock waste, this corn is now a subject of great practical interest to seed producers and U.S. agriculture. This project addresses the potential benefits of these low phytate grains in human nutrition. Test meals of low phytate corn will be fed as polenta and/or grits to healthy adult volunteer women and the absorption of totally safe zinc tracers (stable zinc isotopes) from this corn will be compared with that from a control corn containing a typical quantity of phytate. In a second series of experiments, the absorption of calcium from tortillas prepared from these grains will be evaluated. This project is relevant to potential long-range improvement in and sustainability of U.S. agriculture, including its impact on global markets.

9900685 Retinoid Regulation of Genes in Hepatic Gluconeogenesis

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Grant 99-35200-7607; $260,251; 3 Years

To understand the role of diet in promoting health, it is imperative to delineate the role of specific nutrients in the human body. To this end, we are seeking to further define the numerous effects of vitamin A on gene expression. Utilizing vitamin A deficient mice that contain a foreign gene that we can monitor, we will measure vitamin A regulation of this gene in liver under different conditions of altered vitamin A status. The studies in this project will also assess the effects of vitamin A on liver glucose metabolism using a recently developed method, *in vivo* liver nuclear magnetic resonance (NMR). We will also examine the cellular vitamin A signaling pathway, to determine how the vitamin A message is sent to the nucleus of the cell and is transmitted to the DNA. We will examine the nuclear vitamin A receptors and their binding to specific regions of the DNA in target genes. Additionally, co-activators and co-repressors which are recruited by vitamin A receptors to the transcriptional machinery of target genes will be measured. As the final objective, we will examine the requirement for vitamin A for the correct differentiation of the liver in the developing mouse fetus. Overall, this project will determine the need for vitamin A in the correct differentiation and function of the liver and endogenous glucose production by this tissue. Potentially, this will increase our understanding of the metabolic dysfunction which occurs in liver in type 2 diabetes, which is characterized by oversecretion of glucose by the liver.

9900795 Dietary Conjugated Linoleic Acid as a Novel Antithrombotic Agent

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Strengthening Award; Grant 99-35200-8580; $130,000; 2 Years

Numerous studies have been carried out to assess the role of dietary fat and fatty acids in the modulation of risk factors for cardiovascular and other diseases. Conjugated linoleic acid (CLA) has recently received considerable attention in view of its chemoprotective properties in animal models with various tumors or atherosclerotic lesions. CLA has been found predominantly in meats and dairy products and is a mixture of several positional and geometric isomers of linoleic acid (LA) containing conjugated double bonds. We have obtained *in vitro* evidence that CLA inhibits both human platelet aggregation and formation of the pro-aggregatory thromboxane A2 and that CLA is more potent than the corresponding nonconjugated LA. To further delineate this potential antithrombotic role for CLA, this proposal will determine whether or not CLA can alter the platelet anti-aggregatory potential of the endothelium. In view of the well-established protective role of prostacyclin in vascular tone and hemostasis, the *in vitro* study will focus on the effect of CLA on 1) endothelial (EC)-induced inhibition of platelet aggregation, 2) EC prostacyclin formation in EC-platelet mixtures and 3) EC prostacyclin formation via both the constitutive as well as inducible-prostanoid pathways. The effects of both CLA and LA will be compared to assess the importance of a conjugated double bond relative to a nonconjugated 1,4-diene functionality. If the proposed research yields additional evidence for an antithrombotic effect of CLA, this could provide justification for inclusion of dietary CLA as a fatty acid component in foods.

9900700 Japanese and White Female Adolescent Maturation

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Strengthening Award; Grant 99-35200-7619; $220,000; 2 Years

Prevention of osteoporosis requires building as much bone as possible during the adolescent period of rapid growth. Delaying the onset of menstruation (and the increased estrogen exposure associated with bone mass development) may reduce breast cancer. Prevention of these two chronic diseases may be in opposition, estrogen being “good” for bone but “bad” for breast cancer. Our objective is to identify modifiable factors in diet and physical activity that simultaneously result in high levels of bone mass development and delayed estrogen exposure associated with the onset of menstruation, and the timing of influence of those factors. Six hundred girls of White and Japanese ethnicity will be studied at Kaiser Permanente clinics in an observational study of factors influencing age of onset of menstruation and bone mass. Dietary (soy, calcium, fiber, fat, protein and energy), physical activity and body size factors influencing pubertal stage, age of onset of menstruation and bone mass will be identified and their dietary, activity and body size characteristics will be compared with those in the lowest level of bone mass and age at onset of menstruation. This study will contribute to the prevention of cancer and osteoporosis by identifying the relative contribution of dietary, physical activity, and body size factors to female adolescent maturation.

9900789 Regulation of Osteoblast Apoptosis by Vitamin D
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Grant 99-35200-7614; $225,000; 3 Years

Osteoporosis is a disease characterized by extensive loss of skeletal tissue which primarily affects post-menopausal women and the elderly. Changes in the amount of skeletal tissue reflect imbalances between loss of bone and formation of new bone. New bone formation is dependent on the number of functional osteoblasts, cells that synthesize and secrete proteins on which minerals, such as calcium, become deposited. Osteoblast cell number reflects both the number of new osteoblasts generated and the numbers of osteoblasts undergoing cell death. Although numerous regulators of osteoblast growth and maturation have been identified, little attention has been directed towards understanding the process of cell death in bone cells. Vitamin D is an important regulator of calcium status and is essential for bone health. Specifically, vitamin D acts directly on osteoblasts to maintain their maturation and function. In this proposal we will examine whether the beneficial effects of vitamin D on bone also involve regulation of the cell death pathway known as apoptosis. We will test the hypothesis that vitamin D acts to suppress osteoblast apoptosis. In preliminary studies, we have demonstrated that pretreatment of bone cells with vitamin D can protect against cell death induced by natural mediators (cytokines) and chemotherapeutic drugs. The proposed experiments will map intracellular pathways to determine at what point vitamin D interferes with the cell death pathway. We anticipate that these studies will identify an important role for vitamin D in maintenance of osteoblast survival, and could provide a mechanistic understanding of the beneficial effects of vitamin D on post-menopausal and/or age-related bone loss.

9900798 Lutein as an Antagonist of the Bioavailability and Bioconversion of Beta-carotene
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Grant 99-35200-7565; $180,000; 2 Years

The abundant beta-carotene in dark green leafy vegetables has low biological availability for conversion to vitamin A. Lutein, a characteristic component of photosynthetic tissues, has a molecular structure similar to that of beta-carotene but cannot be cleaved in the small intestine or other tissues to produce vitamin A. Based upon evidence that includes our previous studies, we have formulated the hypothesis that lutein is the component in dark green leafy vegetables that restricts the absorption and subsequent conversion of beta-carotene to vitamin A in humans. This project will test our hypothesis by using beta-carotene and lutein labeled with carbon 13 (a stable isotope that will allow us to trace absorption and conversion of beta-carotene to vitamin A) in small doses that simulate the amounts and proportions absorbed from the digesta of dark green leaves. Human subjects will ingest the following carbon 13-labeled doses in random order: 1 milligram beta-carotene, 1 milligram beta-carotene plus 1 milligram lutein, 1 milligram beta-carotene plus 3 milligrams lutein. The intestinal absorption of carbon 13-labeled beta-carotene and lutein and the production of carbon 13-labeled retinyl esters (vitamin A) will be measured in the triacylglycerol-rich lipoprotein (TRL) fraction of plasma by using gas chromatography-combustion interfaced-isotope ratio mass spectrometry. The TRL fraction is used as a vehicle to assess intestinal absorption of beta-carotene in humans. This research is expected to provide a basis for agricultural strategies to ensure that the beta-carotene in vegetables is biologically available to produce a sustainable, global solution to vitamin A deficiency.

9900682 Role of Vitamin A in Lung Epithelial Cell Repair
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Grant 99-35200-7602; $170,000; 2 Years

The importance of vitamin A in preserving lung tissue integrity and function is well established, but the mechanisms involved have not been defined clearly. The objective of this proposed research is to improve our understanding of how vitamin A protects the lung against injury and diseases. The lung cell that is involved in cell repair is called the type II pneumocyte. This specific cell type depends upon vitamin A in order to initiate the repair process and restore the lung tissue. The precise mechanism for this important role of vitamin A is not known. In the proposed research we plan to investigate one potential mechanism which involves positively charged molecules called polyamines. These polyamines are required for normal cell growth during lung development and for cell repair after toxin-induced injury. We plan to measure the synthesis and transport of polyamines by the lung cell in vitamin A deficient rats, as well as in rats that had been treated with an environmental toxin called monocrotaline. Half of the monocrotaline-treated rats will be given additional vitamin A to protect the lung from this environmental toxin. In both the vitamin A deficient and monocrotalin-treated rats we will evaluate the role of vitamin A and its interaction with polyamines. The goal of this research is to enhance our understanding of how vitamin A is used by the lung to repair itself.

9900816 Stimulator of Iron Transport Function and Regulation in Intestinal Iron Uptake

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Grant 99-35200-7581; $210,000; 2 Years

Iron is absolutely essential to maintain human health. Iron deficiency can lead to profound anemias while iron overload causes many pathological disorders due to the mineral's toxicity. Iron absorption is therefore tightly regulated in response to the body's demands. To promote optimal health, there is a need to understand the cellular and molecular mechanisms that influence nutritional status and the absorption of iron. However, very little is known about the uptake of iron across biological membranes, and more specifically, its import across the intestine. Our laboratory recently discovered a protein called SFT for Stimulator of Iron (Fe) Transport. SFT functions in the membrane transport of iron. While we know that SFT is expressed or synthesized in the intestine, we understand very little about its function in dietary iron absorption. To investigate SFT function and regulation, we propose to study how it mediates iron transport in a human intestinal cell line called Caco-2. To explore how nutritional status may regulate the appearance of SFT in the intestine, we will also study its pattern of expression in anemic mice. A prediction is that if SFT is involved in dietary iron absorption, then expression of this iron transport protein may be upregulated in anemic animals when the demand is high. Finally, we will study the regulation of SFT expression at the genetic level to identify factors that may interact to stimulate synthesis of SFT and dietary iron absorption.

9900695 Vitamin A Activity of Vegetable Carotenoids in Men

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Grant 99-35200-7564; $190,000; 2 Years

The objective of this multi-disciplinary team study is to define the vitamin A value of spinach and carrots in well-nourished adult males. As a major and safe vitamin A source for a vast population in the world, it is essential to determine the conversion efficiency of provitamin A carotenoids to vitamin A. Our objective is of importance as a step in planning rational vitamin A deficiency prevention programs and for accurately determining the vitamin A recommended dietary allowance in well nourished populations. This investigation uses intrinsically labeled spinach, carrots, and a standard labeled vitamin A to evaluate the bioavailability and the bioconversion of provitamin A carotenoids to vitamin A. We will first produce hydroponic spinach and carrots from heavy water and then feed the deuterated spinach or carrots to volunteers. We will determine 1) blood response of labeled carotenoids and labeled vitamin A derived after the labeled vegetable meal and 2) the vitamin A response from a standard labeled vitamin A dose by advanced mass spectrometric techniques. Using the labeled standard vitamin A as a vitamin A reference, the conversion of spinach and carrot provitamin A carotenoids to vitamin A will be estimated by comparing the two forms of labeled vitamin A (one from the vegetables and another from the standard) in serum. Therefore, the vitamin A value of dietary spinach and carrots in well-nourished adult males will be determined.

9900809 Effect of Vitamin K Depletion and Repletion in Elderly Women on Calcium and Bone Metabolism

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Grant 99-35200-7583; $235,000; 3 Years

Osteoporosis is an important public health problem affecting millions of elderly persons in the United States with associated health costs in excess of $20 billion per year. Identification of modifiable dietary factors that could influence bone mineral density and osteoporotic fracture risk could lead to important low-cost osteoporosis prevention strategies and encourage consumer food consumption patterns related to green leafy vegetables, a rich source of dietary vitamin K. Mounting evidence from various studies has implicated vitamin K status as a potentially important nutritional factor that is associated with both bone mineral density and the risk of bone fracture. However, to date, there has been no direct demonstration in humans that dietary vitamin K depletion and repletion influence calcium or bone metabolism. We recently found that dietary vitamin K depletion can have a significant effect on bone metabolism in young adults. Thus, we propose to investigate the effects of dietary vitamin K depletion and repletion on calcium and bone metabolism in elderly women, the primary subpopulation at risk of developing bone fractures. We hypothesize that suboptimal vitamin K status will have an adverse effect on bone metabolism in otherwise healthy elderly women. The long-term goal of our investigations is to define the optimal level of vitamin K intake to minimize bone loss and thereby contribute to a reduction in the risk of osteoporotic fracture. In addition, this study will provide important information to evaluate the adequacy of the current Recommended Dietary Allowance (RDA) for vitamin K in the elderly.

9900903 Decreased Oxidation of Eicosapentaenoic Acid/Docosahexaenoic Acid-Enriched Low-density Lipoprotein: Possible Protective Effect Against Atherosclerosis

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Grant 99-35200-7784; $111,336; 1 Year

Cardiovascular disease (CVD) remains the leading cause of death in the United States. It is especially prevalent in postmenopausal women. Efforts to improve these statistics are many but foremost among them is dietary fat modification. Dietary guidelines encourage Americans to increase their intake of polyunsaturated fat but decrease their intake of saturated fats. These guidelines are based on changes in serum cholesterol, the dominant biomarker of CVD at the time that the guidelines were developed. An unexpected result of increasing the intake of polyunsaturated fat is that an imbalance in the kinds of polyunsaturated is produced. The content of (n-6) fatty acids, those found in high concentrations in corn and soybean oil, is increased while that of the (n-3) fatty acids, such as are found in fish, is decreased. The ramifications of this imbalance are not clear but the (n-6) fatty acids tend to be inflammatory while the (n-3) fatty acids are anti-inflammatory. The purpose of this study is to investigate the impact of different concentrations of the (n-3) fatty acids eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in postmenopausal women. Studies such as this are needed to provide information necessary for determining public health policy of the different kinds of polyunsaturated fatty acids.

9900702 Federation of American Societies for Experimental Biology Summer Research Conference on Calcium Oxalate in Biological Systems

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Grant 99-35200-7592; $5,000; 1 Year

Funds were requested to provide partial support for a FASEB Summer Research Conference entitled Calcium Oxalate in Biological Systems to be held at Copper Mountain, CO, on August 15-20, 1999. This conference will bring together basic scientists and clinician scientists from a variety of disciplines whose research deals with calcium or oxalate or some aspect of their interaction to form crystals. Such scientists will include plant biologists who investigate the orderly formation of calcium oxalate crystals of precise shape in specialized plant cells, microbiologists who examine the ability of microorganisms to metabolize oxalate, nutritionists who study the absorption and bioavailability of dietary calcium and oxalate, physiologists who examine the transport of calcium and oxalate and their interaction with organs, biochemists and cell biologists who investigate aspects of their intracellular function and metabolism, and clinical scientists who examine the pathophysiology of calcium oxalate crystallization, particularly in the formation of kidney stones. The goals of the Conference are: 1) to identify issues of consensus and controversy; 2) to promote a cross-fertilization of ideas; 3) to provide opportunities that facilitate scientific collaborations; and 4) to promote scientific exchange of information, ideas and methodologies. Nine scientific sessions will be held over a 5 day period. Questions that should arise in the conference include the functional role of oxalate and calcium oxalate in plants, environmental factors that influence calcium and oxalate levels in plants, the bioavailability of these plant components for absorption, and the nature of the absorption processes.

9900693 The Function of Post Prandial Vitamin A in Retinol-binding Protein Deficiency

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Grant 99-35200-7611; $275,000; 3 Years

We have disrupted the mouse retinol-binding protein (RBP) gene using gene targeting techniques. RBP-deficient mice, although viable and fertile, have significantly reduced blood retinol (vitamin A) levels. We are proposing basic studies of vitamin A transport and metabolism using this novel animal model. Since RBP is thought to be the sole transporter for retinol in the blood, the existence of RBP-deficient mice raises a question as to how the tissues of mice lacking RBP obtain the vitamin A they need to maintain normal vitamin A-dependent functions. We believe that tissues can acquire vitamin A directly from chylomicron or chylomicron remnant retinyl ester (dietary vitamin A). This hypothesis was reached from other studies that we carried out exploring the effects of lipoprotein lipase (LPL) activity on chylomicron retinyl ester clearance from the circulation. In these studies, we observed that the level of LPL expression in some extrahepatic tissues directly correlated with the amount of postprandial vitamin A taken up by tissues. It has been known since the mid-1960s that approximately 25% of dietary vitamin A remains associated with peripheral tissues and is not cleared by the liver; the significance of this observation was not understood. Using RBP-deficient mice, we will investigate whether the uptake of dietary vitamin A (at levels that might be encountered in “normal” diets) contributes to extrahepatic tissue vitamin A pools in both a quantitatively and functionally significant manner. Upon completion, our studies will provide new understanding of vitamin A transport and metabolism by exploring cellular and molecular mechanisms that underlie dietary requirements for vitamin A.

9900707 Regulation of the Mobilization of Stored Vitamin A
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Postdoctoral Fellowship; Grant 99-35200-7612; $90,000; 2 Years

Despite the extreme day-to-day variability in dietary vitamin A intake, relatively constant levels of retinol are maintained in the circulation. All circulating retinol is transported in the blood bound to its specific transport protein, retinol-binding protein (RBP). It is generally accepted that hepatocytes represent the major source of RBP-retinol, and that RBP-retinol release from hepatocytes is responsible for maintaining constant serum RBP-retinol levels. The mechanisms responsible for regulating mobilization of hepatic RBP-retinol stores is not well characterized. However, it is known that RBP secretion by hepatocytes is dependent on retinol availability. Under conditions of vitamin A-deficiency, RBP accumulates in the endoplasmic reticulum (ER) of hepatocytes, and upon addition of retinol, accumulated RBP is rapidly secreted. Since RBP mRNA levels remain fairly constant under most conditions, it is likely that regulation occurs post-translationally. Like RBP, apolipoprotein B100 (apoB) is a secretory protein which is synthesized in hepatocytes in great excess of that which is secreted, and which is dependent upon lipid binding for secretion. RBP secretion may mimic apoB secretion, and in fact, data available to date suggest that they share common pathways and/or chaperone protein interactions. The long-term goals of this proposal are to carry out experiments to enhance our understanding of the regulation of RBP-retinol secretion by hepatocytes. These studies will be modeled on studies which have explored the regulation of apoB secretion. A greater understanding of retinol-RBP secretion will have a marked impact on our understanding of whole body vitamin A metabolism.

9900814 Obesity and the Initiation of Lactation
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Grant 99-35200-7708; $240,000; 2 Years

The goal of the proposed research is to contribute to our understanding of how maternal fatness at the time of conception affects biological factors that are important for the successful initiation of lactation. To that end, we will conduct a preliminary study in 20 women who are of normal weight and 20 women who are obese at the time of conception. Subjects who plan to breast-feed will be identified in the obstetric clinic of Bassett Hospital in Cooperstown, NY. We will enroll the subjects during the last trimester of their pregnancy and we will study them 2-3 days after they deliver their babies. At this time, we will take a blood sample to measure the concentration of glucose, insulin and various other metabolites and hormones. We will take a series of blood samples in the two hours after a nursing episode to determine how prolactin concentrations change in response to suckling. Mothers will also weigh their babies before and after every breast-feeding for the next 24 hours to determine how much milk they are consuming. Increasing the number of women who are able to breast-feed their infants successfully is an important national health goal and an important goal of the Special Supplemental Nutrition Program for Women, Infants and Children (WIC), which is run by USDA. This research will contribute to achieving these goals.

9900680 Soy Isoflavones and Vitamin E Dose-Dependently Improve Fracture Healing in the Rat Model
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Grant 99-35200-7606; $240,000; 2 Years

A conservative estimate in the United States alone is that nearly half of all women over the age of 50 will suffer a fracture related to osteoporosis, resulting in more than 1.5 million fractures a year, including painful vertebral fractures. Our preliminary findings indicate that soy isoflavones and vitamin E have the potential to promote the healing process in fractured bone. The aim of this study is to evaluate the dose-dependent effects of vitamin E, soy isoflavones, and their combination on bone quality and the fracture healing process in a fracture-repair osteopenic rat model. The study will utilize 190 12-month-old female rats divided into 19 groups of 10 rats each. Rats will be sham-operated or ovariectomized to induce bone loss for a period of 120 days. Thereafter, a bone fracture-repair model in the rat will be created and dietary treatments will follow immediately. After 60 days, improvements in fracture healing, bone mass, and bone strength will be assessed. Biochemical markers of bone turnover as well as the formation of bone resorbing cells originating from marrow will be evaluated. If soy isoflavones and vitamin E independently or synergistically promote bone quality and facilitate fracture healing, these findings would be of critical importance as a basis for making dietary recommendations for human skeletal health.

9900706 Iron Deficiency Anemia and Cognitive Performance in Young Women

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Grant 99-35200-7610; $140,000; 2 Years

Iron deficiency is known to affect the lives of more than 1.2 billion people worldwide; many of these individuals are women, children, and infants. While there is strong evidence that young children are at significant risk, only recently have scientists begun to question whether or not iron deficient adolescents and young adults also have cognitive or behavioral dysfunction as a result of the nutrient deficiency. It is our contention, based on a recent report in the medical literature, and our preliminary data, that it is likely that adolescents and adults are also at risk for attentional and memory dysfunction due to iron deficiency. Since the published data demonstrate that non-anemic iron deficient individuals are affected (approximately 8-15% of reproductive age women in the US population), it seems critical to define the relationship of the severity of the iron deficit to the severity of the mental dysfunction. The overall aim of the proposed study is to examine the effects of iron deficiency on cognitive and behavioral performance in young women. We will use a placebo controlled intervention trial where 50 anemic subjects will receive iron supplements and 50 anemic subjects will receive a placebo supplement for a four month period. We will test them before and after this intervention period. The specific aims of the project are to demonstrate that iron deficiency anemia is associated with decreased attention and less behavioral "interaction" in young women. We also want to demonstrate that an iron intervention will correct the mineral deficiency and lead to an improvement in measures of cognition and behavior.

9900655 Mechanisms of Endothelial Cell Dysfunction During Selenium Deficiency

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Grant 99-35200-7570; $240,0000; 3 Years

Disruption of endothelial cell function was suggested to be a primary event leading to both acute and chronic disease processes such as atherosclerosis, thrombosis, and peripheral vascular disease. It is well established that overall nutritional status of the host will influence endothelial cell integrity and consequently impact the development of certain pathological conditions. Although selenium status is crucial to the development of vascular dysfunction, the exact mechanisms by which this micronutrient are able to influence endothelial cell metabolism is not known. The long term goal of this research is to elucidate the mechanisms involved in endothelial cell dysfunction which occurs as a result of inadequate selenium nutrition. We propose to investigate the effects of altered selenium status on Nuclear Factor (NF)-kappa B activation in endothelial cells and relate its induction to both proinflammatory and protective gene responses of the vascular endothelium. This work will provide information concerning the molecular basis of how selenium deficiency can influence resistance to metabolic vascular disorders.

9900802 Vitamin A and Sympathetic Neuronal Development

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Grant 99-35200-7604; $150,000; 2 Years

Vitamin A plays an essential role in the developing nervous system. Provision of either excess or insufficient amounts of vitamin A during critical periods of embryonic development can lead to nervous system abnormalities. Studies in this research proposal are directed at understanding how the vitamin A metabolite, all-*trans* retinoic acid, functions in the developing sympathetic nervous system of the embryonic chick. Studies of cultured embryonic chick sympathetic neurons reveal an essential role for all-*trans* retinoic acid in both neuronal survival and differentiation. All-*trans* retinoic acid exerts its effects by binding to nuclear retinoic acid receptor proteins which, in turn, regulate gene expression. The objective of this research is to define the changes in gene expression that follow the binding of all-*trans* retinoic acid to its receptors. This will be accomplished by identifying the first wave of early immediate genes that are induced or repressed after exposure of embryonic sympathetic neurons to all-*trans* retinoic acid. Once identified, experiments will be conducted to determine whether these gene products play essential roles in either neuronal survival and/or neurite outgrowth. The results of this research will enhance our understanding of how vitamin A contributes to the formation of a normal nervous system. The goals of this research are central both the USDA mission to understand mechanisms underlying the relationship between diet and health (e.g. influence of dietary components on the nervous system) and the mission to understand the cellular and molecular mechanisms whereby dietary components influence gene expression.

ENSURING FOOD SAFETY

Panel Manager - Dr. Susan Sumner, Virginia Polytechnic Institute and State University
Program Director - Dr. Etta Saltos

Safety of food products is of paramount importance to the producer, processor, distributor, and consumer. The research program in food safety focuses on research questions involving disease-causing microorganisms, their products, naturally occurring toxicants which contaminate food. The program emphasizes detection, prevention and control. Projects may focus either on pre- or post-harvest/slaughter origin of the microbial agent or toxicant. Additionally, the program supports research of the identification of obstacles to adopting safe food habits, with particular emphasis on factors affecting consumer attitudes and behavior, as well as the development of intervention strategies to improve food safety habits.

9902774 Irradiation in a Combination Approach to Enhance Vegetable Safety

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Strengthening Award; Grant 99-35201-8554; $128,000; 2 Years

Fresh-cut fruits and vegetables are often considered among the most healthful and safe foodstuffs, yet new disease-causing microorganisms continue to emerge and an increasing number of outbreaks are being traced to fresh produce. With the understanding that no single preservation technique will effectively destroy pathogens while maintaining quality, our objective is to determine the optimum levels of irradiation, modified atmosphere packaging (MAP), and chlorination that will enhance safety while preserving sensory attributes such as texture, color, and flavor. Selected products include shredded iceberg lettuce and shredded cabbage and other minimally processed vegetables in which the cut surfaces provide a substrate for the proliferation of spoilage and pathogenic microorganisms. Conventional sanitizing procedures are inadequate, however, harsher treatments can affect fresh-like quality. Because irradiation has the ability to penetrate inside cells, it can be highly effective to treat these products. Additionally, chlorination can destroy surface contamination while MAP can extend storage time. Together these techniques can reduce overall levels of microorganisms and hence enhance safety with the added benefit of extending shelf-life. In the absence of immediately applicable solutions, this project will reveal the efficacy of combination processing to address the emerging safety issues associated with minimally processed vegetables. The added security, as well as the economic benefit of shelf-life extension, should encourage consumers and processors to utilize this novel combination of accepted and proven technologies.

9902763 Recalcitrance of *Clostridium perfringens* to High Hydrostatic Pressure Processing

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Grant 99-35201-8608; $95,000; 2 Years

The overall project goal is to obtain an accurate assessment of the response of the foodborne pathogen, *Clostridium perfringens*, to high hydrostatic pressure in order to better understand the mechanism of inactivation and identify those controllable factors most important in protection of this pathogen from the effects of high pressure processing in foods. A novel technology, the pressure processing of foods offers U.S. agriculture a commercially viable non-thermal means, i.e., "cold pasteurization", for the processing of foods and beverages resulting in longer shelf-life and improved safety while maintaining sensory characteristics and nutrient content nearly identical to fresh or raw products. In light of the mounting concerns regarding the safety of chilled meats and meat products, and the use of irradiation as a process for the treatment of foods, optimization of high hydrostatic pressure as a food processing technique carries significant potential benefit as this technology matures and is applied to refrigerated commodities in national and global markets. *C. perfringens* is one of the most common causes of food-related diarrheal diseases in the U.S. Spores, such as those produced by *C. perfringens*, have been shown very resistant to applications of high pressure. In other sporeforming bacteria, it is known that pressure resistance is significantly affected by the temperature of sporulation. Allied processing factors known to protect bacterial endospores include environments of reduced water activity, partial germination of spores due to exposure to different levels of pressure, extended initiation of germination caused by pH, and the protective effect of food itself. These factors will be examined in this study.

9902786 Are Virulent Strain-Specific DNA Sequences of *Vibrio vulnificus* Essential For Virulence?

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Grant 99-35201-8606; $150,000; 2 Years

Vibrio vulnificus is the leading cause of death in the U.S. associated with consumption of shellfish. The rapid and lethal disease that can follow consumption of contaminated oysters has had immense negative impact on the industry as well as consumer attitudes and practices. However, not all strains of *V.vulnificus* have the potential to cause disease; there are

virulent and avirulent strains in the environment. Currently there is no practical test to determine if seafood products contain hazardous versus avirulent strains of *V.vulnificus*. In currently funded USDA research, we are using genetic methods to identify DNA sequences that are unique to the virulent versus avirulent strains. These virulent strain-specific sequences could be used to screen oysters and harvested waters for the presence of *V.vulnificus* strains with the potential to cause human disease. We propose to examine the relationship of virulent strain-specific DNA sequences to the disease process by determining if genes encoded on virulent strain-specific genomic sequences are essential for virulence, or if they are only markers for virulence by coincidence. Determining the functional importance of DNA sequences unique to virulent strains will add credibility to the use of these new tools to accurately screen for virulent versus avirulent strains. By identifying virulence genes, we will contribute to understanding how this devastating food-borne pathogen can kill humans so rapidly after consumption of contaminated sea food. This information could be integrated into CDC, FDA, and State efforts to determine the epidemiology of infections and to develop interventions to reduce risk of *V.vulnificus* disease.

9902875 Inactivation of Pathogens on Alfalfa Seeds

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Grant 99-35201-8613; $114,319; 2 Years

From 1995 to 1998, eleven reported outbreaks involving about 1200 cases of *Escherichia coli* O157:H7 and *Salmonella* infections in the U.S. have been associated with consumption of alfalfa sprouts. The overall goal of this research is to develop a procedure to kill *E. coli* O157:H7 and *Salmonella* on alfalfa seeds without reducing the ability of the seeds to germinate and produce sprouts. Objectives are to determine: 1) the effectiveness of combinations of detergents and disinfectants in killing these pathogens; 2) the effectiveness of simultaneous application of heat and detergents/disinfectants on inactivation of these pathogens; 3) the efficacy of these treatments in eliminating pathogens on various types of alfalfa seeds; and 4) the ability of *E. coli* O157:H7 and *Salmonella* to survive on alfalfa seeds as influenced by temperature and relative humidity. Detergents to be evaluated include those exempted by the U.S. Environmental Protection Agency and the Food and Drug Administration from requirement of tolerance and are generally recognized as safe (GRAS). Disinfectants to be tested will include those known to be effective in removing microorganisms from raw fruits and vegetables. Effectiveness of treatments in killing *E. coli* O157:H7 and *Salmonella* at temperatures ranging from 70 to 175°F will be determined. Survival of these pathogens on alfalfa seeds stored at 40 to 140°F for up to 2 years will be monitored. Viability of seeds as affected by all test treatments will be determined. Information gained from this research will be valuable in developing strategies to eliminate *E. coli* O157:H7 and *Salmonella* from alfalfa seeds as well as other seeds intended for sprout production, thus greatly minimizing the risk of illness associated with eating of raw seed sprouts.

9902833 Improving Pathogen Decontamination Treatments for Fresh Produce

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Grant 99-35201-8184; $185,000; 2 Years

In recent years fresh produce has been involved in numerous disease outbreaks. Currently there is no effective intervention step that will remove or inactivate pathogenic bacteria that may be on the surface of fresh produce. Our previous research indicates that pathogenic bacteria can enter plant tissue stomata, cracks in the waxy cuticle, and damaged tissue. At these sites they are protected from the effects of aqueous chlorine solutions. Bacteria not removed by an initial wash may be coated with wax or oil when the produce is prepared for distribution. After this point, the effect of washing by consumers or food service establishments on pathogen levels is unknown. We propose that effective decontamination treatments will be those that remove or inactivate pathogenic bacteria at protected locations on the produce surface. This research will determine the role of stomata, damaged tissue, waxy cuticle, and applied waxes and oils in protecting pathogenic bacteria from removal or inactivation on plant surfaces. Strategies for defeating these protective mechanisms will be evaluated. Such strategies will involve application of sanitizing agents containing food grade solvents or surfactants that may penetrate to protected sites and provide an effective decontamination treatment. Results of this research will provide basic information necessary for the commercial development of fresh produce decontamination treatments.

9902824 Genetic Markers and Pathogenesis Features of *Listeria monocytogenes* serotype 4b

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Grant 99-35201-8183; $220,000; 3 Years

Listeria monocytogenes is a troublesome food-borne bacterial pathogen because of its ubiquitous distribution, its ability to grow in refrigerated foods, and its involvement in severe and often fatal illness (listeriosis). Pregnancy, immunosuppression, and extremes of age are predisposing conditions, and ready-to-eat, refrigerated foods have been frequently implicated in infection. Contamination of ready-to-eat foods by *Listeria*, including dairy and meat products, also

accounts for numerous and costly food recalls, thus rendering the microorganism a severe threat both to public health and to agriculture. Although many different strains of *Listeria monocytogenes* can contaminate food, not all appear to be equally capable of causing disease. A small number of antigenic types (serotypes) account for the majority of strains implicated in human illness. Of these, serotype 4b is of special interest, being involved in almost all common-source outbreaks of listeriosis and in numerous sporadic cases. We have identified two gene clusters unique to serotype 4b strains and essential for the expression of serotype-specific antigenic components on the bacterial cell wall. We propose to complete the molecular characterization of these regions in order to gain novel information on the serotype-specific genes and in order to identify DNA-based diagnostic reagents for this serotype. We will also investigate the possible involvement of serotype-specific determinants in pathogenesis, using endothelial cells as a biologically relevant model system. Such investigations may yield novel molecular reagents that will facilitate monitoring and detection of clinically important strains of the pathogen, and may further elucidate the virulence attributes of such strains.

9902843 Identifying Factors that Promote Clearance of *E. coli* O157:H7 From Cattle

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Grant 99-35201-8539; $265,000; 2 Years

Escherichia coli O157:H7 foodborne illness results in hemorrhagic colitis that can lead to life-threatening complications. Human disease has followed consumption of contaminated under-cooked ground beef, unpasteurized dairy products, vegetables, unpasteurized apple cider, drinking water or recreational water. Healthy cattle carry *E. coli* O157:H7 into processing plants and disperse this human pathogen in the environment. Decreasing the incidence of *E. coli* O157:H7-positive cattle will decrease the incidence of human disease. The average duration an individual animal is culture-positive for *E. coli* O157:H7 is 30 days, but the ranges in duration individual animals are culture-positive vary from a few days to one year. The conditions that induce acquisition of *E. coli* O157:H7, prolong or curtail its presence, and cause its clearance from the ruminant gastrointestinal tract (GIT) are not understood. This research will test the hypothesis that cattle diet and colonic cell proliferation may be used in pre-harvest interventions to promote the clearance of *E. coli* O157:H7 from the GIT of cattle. Our first objective is to expand our observations that grain diets promote clearance of *E. coli* O157:H7 from the ruminant gastrointestinal tract and determine the natural mechanism(s) that influence the clearance of these bacteria. Our second objective is to confirm our preliminary data that epithelial cell proliferation in the lower GIT promotes rapid clearance of *E. coli* O157:H7 from ruminants. This research directly contributes to the USDA NRICGP Food Safety program goals of decreasing the incidence of food-borne illness by increasing our understanding of the ecology of *E. coli* O157:H7.

9902760 Molecular Mechanisms of Psychrotrophy in *Listeria monocytogenes*

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Grant 99-35201-8607; $253,000; 3 Years

Listeria monocytogenes is a foodborne pathogenic bacterium that causes listeriosis, a disease encompassing meningitis, abortions, and stillbirths, which has a fatality rate of about 25%. This bacterium has the unusual ability to grow at refrigeration temperatures. The highest risk foods for listeriosis are ready to eat and stored at refrigeration temperatures for long periods, and are contaminated with high levels of the bacterium. Food producers incur considerable costs due to product recalls of contaminated foods. A recent high profile multi-state outbreak involving several deaths was traced to cold cuts from a meat producing plant in Michigan. We are studying the membrane fluidity of *L. monocytogenes*, and the genes and proteins involved in growth of the organism at low temperatures. Using the technique of electron paramagnetic resonance we will examine the hypothesis that even at low temperatures there is a fluid region in the membrane consisting of fatty acids surrounding membrane proteins. Novel genes involved in low temperature growth will be identified through differences in gene expression (messenger RNA production) at low temperatures. Proteins produced in elevated amounts in response to low temperatures will be identified using 2-dimensional electrophoresis. The proposed research aims to: 1) increase our understanding of an important foodborne psychrotrophic (cold-growing) pathogen and how it resists the food preservation strategy of chilling; and 2) provide the scientific basis for improved control strategies for *L. monocytogenes*.

9902831 Molecular Biology of Fumonisin Biosynthesis in *Gibberella fujikuroi*

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Grant 99-35201-8124; $100,000; 2 Years

Fumonisins are toxins produced in corn by the fungus *Gibberella fujikuroi*. Evidence has linked fumonisin with cancer in humans. With the likelihood of fumonisin concentrations being regulated world wide, the impact on the U.S. food industry will be substantial. The long-range goals of this research are to understand fumonisin biosynthesis and to develop

novel approaches for eliminating fumonisin contamination of food sources. We have shown that fumonisin biosynthesis is suppressed by ammonium. The data suggest that targeting the regulation of nitrogen metabolism may be a reasonable strategy to minimize fumonisin contamination. The specific objectives are to: 1) use molecular techniques to isolate genes with expression patterns that correlate with fumonisin biosynthesis, and 2) isolate tagged strains of the fungus with mutations affecting the regulation of fumonisin biosynthesis. We anticipate that mutations affecting fumonisin production will involve genes that encode regulatory factors that may be common to several pathways regulated by nitrogen metabolism. Information from this research will further our understanding of the genetics and molecular biology of fumonisin biosynthesis which should give insight to new approaches for eliminating fumonisin contamination.

9902848 Ecological Distribution of *E. coli* O157:H7 Strains in Agricultural Environments

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Grant 99-35201-8610; $210,000; 2 Years

E. coli O157:H7 has emerged as a significant health concern, causing illness and death in humans. The source of this pathogen is usually related to contaminated feeds, including beef products. Control of *E. coli* in the beef industry will require a concerted effort from all segments of the industry, including the farm level. However, the development of effective on-farm control schemes for this pathogen require an understanding of the natural ecology of *E. coli* O157:H7 which is presently unknown. It is known that different genotypes of *E. coli* O157:H7 exist. The goal of this project is to determine the distribution of genetically identified *E. coli* O157:H7 isolates in agricultural environments, as a means to identifying the sources of infection for cattle. This information will allow for the development of land management and water-use practices and policies for the control and/or reduction in the overall prevalence of *E. coli* O157:H7 in agricultural food products. The specific objectives of this project are to quantify the degree of genetic homogeneity of *E. coli* O157:H7 isolates recovered from water sources, wildlife, and cattle feces in agricultural range environments, and to define the distributional patterns of *E.coli* O157:H7 in agricultural range environments. *E. coli* O157:H7 isolates obtained from field-based sampling of cattle, wildlife and water will be genetically defined using pulsed-field gel electrophoresis and high resolution genome scanning. We will then compare the degree of genetic similarity of E.coli O157:H7 strains from cattle, water, and wildlife within and between agricultural areas.

9902829 Characterization of Multiple Fluoroquinolone Resistance Among Bacterial Pathogens

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Grant 99-35201-8590; $157,000; 2 Years

In the past few years, strains of *Escherichia coli*, *Salmonella*, and even *Campylobacter* (animal and human origin) have become increasingly resistant to most frontline antibiotics, including third generation cephalosporins, aminoglycosides, and even fluoroquinolones. Infections caused by antibiotic-resistant bacteria are a major and costly human and animal health problem. These infections prolong illness and if not treated in time with more expensive, alternative antimicrobial agents, can lead to increased disease and even death. The use of fluoroquinolones in veterinary medicine continues to polarize the veterinary and medical professions. The controversy is primarily due to the lack of convincing data concerning characterization of fluoroquinolone resistance among animal bacterial isolates. For the United States, there are very few data on the use of fluoroquinolones in animals, the emergence of fluoroquinolone resistant bacteria from animals, and their subsequent emergence in humans. Our specific research objectives are to: 1) Characterize the genetic resistant determinants responsible for fluoroquinolone resistance among veterinary isolates of *E. coli* and *Salmonella* spp.; 2) Determine if veterinary fluoroquinolone use selects for bacterial isolates that are cross resistant to human therapeutic fluoroquinolones; and 3) Determine if the emergence of fluoroquinolone resistance among veterinary and foodborne bacterial pathogens is genetically related. These data shall yield important insights into the molecular mechanisms responsible for bacterial resistance to this powerful class of antimicrobials among veterinary *E. coli* and *Salmonella* strains. Further, the data will also determine if there are potential public health implications regarding the use of fluoroquinolones in animals in the U.S.

9902821 Detection of Viable Enterohemorrhagic *Escherichia coli* using Polymerase Chain Reaction and RNA-based Polymerase Chain Reaction

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New Investigator Award; Grant 99-35201-8125; $111,000; 2 Years

Development of strategies to ensure the safety of food is critical for sustaining U.S. agriculture. Recently developed detection methods based on DNA amplification, termed polymerase chain reaction (PCR), offer a high degree of speed, sensitivity and specificity for pathogen screening in foods. Positive results, however, must still be confirmed by

time-consuming conventional methods as PCR does not differentiate viable from nonviable cells. Methodologies will be developed to rapidly detect viable enterohemorrhagic *Escherichia coli* (EHEC). A new DNA-based detection method, quantitative competitive PCR (QC-PCR), and an RNA-based detection method, RT-PCR of mRNA, will be developed. The *slt-I* and *slt-II* toxin genes will be used as targets. For QC-PCR, a segment of competing DNA similar to the target DNA will be created. A known constant amount of competing DNA is then added to dilutions of extracted target DNA followed by PCR amplification. The amount of target DNA is quantified by a generated standard curve. QC-PCR at two points during enrichment will be used to determine viability. For RT-PCR, mRNA , which is a known indicator of cell viability, will be used. RT-PCR amplification of *slt* mRNA will be used after a brief enrichment to determine viability. The sensitivity of both methods with respect to viable and injured cells will be evaluated. The methods will be applied to artificially contaminated ground beef samples. The development of nucleic acid based methodologies to detect viable EHEC will be applicable to other foodborne pathogens and will provide a more rapid option for monitoring food safety.

9902780 Modeling Bacterial Pathogen/Biocontrol Competition With Changing Temperature

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Grant 99-35201-8682; $117,369; 2 Years

This proposal addresses the President's National Food Safety Initiative to predict and prevent the growth of harmful bacteria in foods. The foods we eat, including fresh fruits and vegetables, contain bacteria. When disease-causing bacteria grow in foods, they must compete with naturally present (generally harmless) bacteria. Current models for bacterial growth in foods do not take into account this microbial competition. Our project is unique in modeling competition between microorganisms. We are developing models for the growth and death of two or more competing microorganisms during changing environmental conditions. This project involves a multi-disciplinary approach to study the competition of microorganisms in foods, including biochemistry, microbiology, mathematics and computer simulation models. The computer simulations and mathematical models being developed will be used primarily as tools to investigate the mechanisms of microbial competition. These models will be validated by experimental observations. One objective of this project is to develop biocontrol strategies for producing safe food products. Our biocontrol approach uses safe bacteria (which are normally used in food fermentations) as food additives; they will be selected for their ability to compete with, and prevent the growth of, pathogens in foods. It is hoped that the principles learned in these studies can be applied to the prediction and prevention of the growth of disease causing bacteria in a variety of foods.

992860 A *Salmonella*-based Vaccine to Prevent *E. coli* O157:H7 Infection in Cattle

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Grant 99-35201-8578; $200,000; 2 Years

Escherichia coli O157:H7 is an emerging zoonotic bacterial pathogen. This strain of *E.coli* causes enteric disease in humans ranging from self-limiting, non-bloody diarrhea to severe hemorrhagic colitis. *E.coli* O157:H7 can also cause devastating extraintestinal illness, most importantly hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). *E.coli* O157:H7 remains by the far the most clinically important enterohemorrhagic *E.coli* (EHEC) serovar at the present time. *E.coli* O157:H7 is a food-borne pathogen. Beef and dairy cattle are thought to be the most important animal reservoir. The most extensive outbreaks of *E.coli* O157:H7 infection have involved ground beef where the destruction of large quantities of contaminated beef products was required. *E.coli* O157:H7 has become a significant human pathogen and has had a major economic impact on the beef industry. Measures to significantly reduce the level of *E.coli* O157:H7 carriage in cattle will ensure microbiologically safer food. We will use several live, attenuated vaccine strains of *Salmonella* expressing important surface proteins of *E.coli* O157:H7 to vaccinated calves. Vaccination of calves with *Salmonella* strains expressing surface proteins of *E.coli* O157:H7 will engender a mucosal immune response. By stimulating mucosal immunity, it is hoped that the *E.coli* O157:H7 will not have an opportunity to colonize the lower bowel of the calves. Calves will then be challenged with virulent *E.coli* O157:H7 and the severity of diarrhea measured. The reduction or elimination of *E.coli* O157:H7 from these vaccinated animals well be determined. Means to significantly reduce or eliminate *E.coli* O157:H7 from cattle will ensure a safer food supply.

9902778 Characterization of Genes Regulating Aflatoxin Biosynthesis

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Grant 99-35201-8073; $220,000; 3 Years

Aflatoxins are toxic and carcinogenic compounds produced in food by the fungus *Aspergillus flavus*. Contamination often occurs before harvest and no effective control procedures are available. The goal of this research is to understand the factors that regulate aflatoxin biosynthesis such that plant gene products or synthesized compounds can be

employed to inhibit aflatoxin formation. One approach to study regulation of aflatoxin biosynthesis is to determine those genes expressed during aflatoxin biosynthesis that are not expressed under conditions unfavorable for aflatoxin formation. Several different physiological conditions known to be favorable or unfavorable for aflatoxin biosynthesis will be compared. DNA from an *A. flavus* cDNA library will be spotted on filters and probed with DNA made from mRNA expressed during conditions either conducive or nonconducive for aflatoxin biosynthesis. Genes that are expressed during aflatoxin biosynthesis but not expressed under conditions unfavorable for aflatoxin biosynthesis will be isolated, sequenced, and their function determined. A second approach to identify aflatoxin regulatory genes will involve generating mutants unable to induce transcription of the pathway specific regulatory gene *aflR*. Presumably, these mutants will have a mutation in genes that regulate *aflR*. To identify the functional copy of the mutated gene(s), the mutant will be transformed with DNA clones from a genomic library of *A. flavus* representing all of the genes of the fungus. The gene that restores function will be sequenced and characterized. Once regulatory genes are identified by the procedures above their profile of expression and order in the regulatory circuit will be determined.

9902784 Extrusion Processing as a Means of Reducing Fusarium Mycotoxins in Cereal Foods

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Grant 99-35201-8612; $128,000; 2 Years

Toxin producing Fusarium molds pose major food safety hazards by invading corn and wheat in the field and producing their toxins in the grain. These toxins are called mycotoxins, and include deoxynivalenol (DON, vomitoxin), zearalenone and fumonisins. Fumonisins have been determined to be carcinogenic in toxicity studies by FDA. These mycotoxins have been found to commonly occur in corn and wheat and have also been found in finished grain-based processed food products. Extrusion processing of cereal grains is used extensively in the food industry to produce many breakfast and snack foods. During extrusion processing ground up grain is mixed with water to form a dough-like material that is forced through a tube of decreasing size by a rotating screw. At the end of the tube or barrel of the extruder is a small opening known as a die through which the hot grain dough, under great pressure, exits the extruder with a sudden release of steam and pressure which causes the cooked dough to puff and dry. During extrusion processing, very high temperatures and pressures along with shearing forces are generated. The goal of this research is to find the optimum conditions of extrusion processing which will destroy and reduce the amounts of deoxynivalenol, zearalenone and fumonisins in contaminated corn and wheat under simulated industrial conditions to render the processed grain-based foods free of these toxins. This project will contribute to the safety of the food supply by improving the safety of cereal-based foods.

9902861 A Membrane Fluidity Model for Sensitivity of Foodborne Pathogens to Preservatives

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Grant 99-35201-8611; $230,405; 3 Years

There is poor understanding of how *Listeria monocytogenes*, a food poisoning bacteria, survives in cold hostile environments once thought to kill bacteria or at least prevent them from growing. The cell membrane is the barrier that protects food poisoning bacteria from preservatives and the environment. This research determines how membrane properties regulate the sensitivity of the bacteria to preservatives, acidity, and cold. We believe that the physical state of the cell membrane regulates antimicrobial sensitivity. Changes in membrane composition modify membrane fluidity. Membrane fluidity determines membrane permeability and sensitivity to those food preservatives which act by violating the membrane's protective properties. This model: Composition determines Fluidity determines Permeability determines Sensitivity to Preservatives; will be tested through the following specific objectives: 1) prove that changes in membrane composition (i.e. makeup) change membrane fluidity in *Listeria monocytogenes* cells grown under different conditions; 2) show that changes in fluidity regulate membrane permeability using model membrane systems; 3) demonstrate that sensitivity to preservatives in laboratory models corresponds to sensitivity of *L. monocytogenes* cells in the real world. A better understanding of how the bacteria survive our attempts to kill them will provide new concepts that can be used to increase the effectiveness of current preservatives and processes, and thus, improve food safety.

9902756 Specific Detection and Typing of *Vibrio parahaemolyticus* serotype O3:K6

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New Investigator Award; Grant 99-35201-8075; $110,065; 2 Years

Vibrio parahaemolyticus is a bacterium that is naturally found in marine waters. Shellfish, which obtain food by filter feeding, easily concentrate such bacteria to high levels. Human infection with *V. parahaemolyticus* is most frequently associated with the consumption of seafood, primarily raw or improperly cooked shellfish. While most strains of *V. parahaemolyticus* are not pathogenic to humans, some strains cause gastroenteritis. Of three U.S. outbreaks of foodborne

disease linked to consumption of raw oysters in 1997 and 1998, two have been attributed to a unique strain of *V. parahaemolyticus* (the O3:K6 serotype). This *V. parahaemolyticus* strain may have an enhanced ability to cause disease. Currently, identification of the O3:K6 serotype requires extensive biochemical and genetic tests, which are both time-consuming and expensive. The development of identification tools capable of rapidly identifying the O3:K6 strain of *V. parahaemolyticus* is a high priority. Therefore, our research aims to enhance the safety of seafood consumed in the US by developing a rapid strategy for detecting pathogenic *V. parahaemolyticus* in foods, specifically focusing on serotype O3:K6 strains. The ability to distinguish between potentially dangerous isolates and the more prevalent nonvirulent *vibrios* will provide critically important tools that will allow public health workers to develop scientifically based guidelines for identifying water sources more likely to cause infection.

9902859 Novel Strategies for Determining Thermal Destruction of *Mycobacterium paratuberculosis*
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Postdoctoral Fellowship; Grant 99-35201-8039; $87,784; 2 Years

The American dairy industry annually loses $120 million to Johne's disease, an incurable bacterial infection in cattle. The infection is caused by the bacterium *Mycobacterium paratuberculosis*. Infected cattle shed *M. paratuberculosis* into milk and feces, posing a possible health risk to humans. Although pasteurization of raw milk kills most spoilage and pathogenic microorganisms, isolation of *M. paratuberculosis* from humans suffering from Crohn's disease, an incurable inflammatory bowel disease with clinical symptoms similar to Johne's disease, suggests a possible causal relationship. Processing times and temperatures that will enhance destruction of *M. paratuberculosis* in milk remain to be established. Current standard culturing techniques for enumerating viable *M. paratuberculosis* cells will detect the presence of low numbers, but are laborious, time-consuming and may not allow detection of sublethally heat injured, viable but non-culturable cells (VNC). The VNC may represent a significant proportion of cells surviving heat treatment. To better establish whether *M. paratuberculosis* cells are killed by pasteurization, we will use *M. paratuberculosis* strains engineered to produce light when viable to test the efficacy of pasteurization and to develop a strategy for screening for the presence of *M. paratuberculosis* in raw and processed milk. At the conclusion of this project, we will have established specific heat treatment parameters for the destruction of *M. paratuberculosis* and will have developed a rapid detection method to improve dairy herd health management.

9902758 Transmission of *Listeria monocytogenes* in Food Systems
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New Investigator Award; Grant 99-35201-8074; $192,000; 3 Years

Listeria monocytogenes is a bacterium capable of causing a serious foodborne disease ("listeriosis") in humans. A 1998-9 listeriosis outbreak in the US affected more than 100 people and killed at least 20 people. An estimated 1,700 cases of human listeriosis occur annually in the U.S. About 20-40% of people infected with this bacterium die. Only a few, genetically distinctive *L. monocytogenes* subtypes appear to be responsible for most listeriosis cases, suggesting that not all subtypes are equally capable of causing human foodborne disease. *L. monocytogenes* is commonly present in the environment; thus preventing contamination of food products with this organism is challenging. In fact, most food recalls due to bacterial contamination are a consequence of the presence of *L .monocytogenes*. Current regulations require the recall of any ready-to-eat food containing more than one *L. monocytogenes* cell/25 g. Therefore, *L. monocytogenes* contamination presents a serious economic concern to food processors. This project is designed to better understand the characteristics of different *L. monocytogenes* subtypes and to gain a better understanding of which specific *L. monocytogenes* subtypes cause human disease and how these types differ from those that cannot cause human disease. We will comprehensively characterize *L. monocytogenes* isolates from humans, animals and foods using both DNA fingerprinting methods and methods for evaluating a strain's ability to cause disease. This work will ultimately contribute to the development of science-based food safety regulations that economically meet public health needs.

9902752 Development and Validation of Instruments to Evaluate Food Safety Education
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Grant 99-35201-8126; $200,000; 2 Years

Consumer education about the basic principles of food safety is an important component of preventing food borne illnesses. Impact is difficult to document because of a lack of valid and reliable evaluation instruments. The goal of this project is to develop and validate an instrument that is suitable for evaluation of food safety educational programs for low-income, low-literacy audiences. This is a population cohort believed to be vulnerable to food safety illnesses because of the high proportion of children and elderly living in poverty. The American food supply is among the safest in the world, but

consumers need to take responsibility for their role in maintaining the safety of food rather than placing the burden only on the food producer or processor. The research objectives are: determine the relationship of intermediate variables (stages of change variables, knowledge, attitudes, awareness, self-efficacy, and intention to change) to the behavioral outcomes related to food safety; evaluate whether self-reported behavior changes are a valid way to assess the behavioral outcomes of food safety education; design, validate and test an instrument that will assess food safety behavior and intermediate variables; pilot test the instrument; and revise as needed and retest. The Stages of Change Model will be used to develop and validate intermediate variables. An audit of food preparation behaviors will be used to validate self-reported behaviors. Face validity and field-testing will be used to test the instrument.

9902902 Optical Biosensor Detection of Food Pathogens Based On Direct Measurement of Antibody/Antigen Binding
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Strengthening Award; Grant 99-35201-8435; $180,000; 2 Years

The demands from consumers for fresh, less processed food has increased the need to ensure microbial safety of these products. This project provides the opportunity for a multi-disciplinary effort to create specific biosensors for rapid and early detection of pathogen contamination. These devices have the potential for specifically selecting food pathogens from among the total microbial load within minutes and measuring the concentration as real-time analysis on site. The goal of this project is to develop new prototype biosensor devices that will consist of either a fiber optic fluorometer and magnetic focusing system or a new evanescent thin-film wave guide for the transducers. One advance will be the creation of an immunosensing element which consists of unique new complexes of antibodies attached to solvatochromic fluorescent dyes. This new dye/antibody complex will be designed to detect cell attachment directly when immobilized on magnetic microspheres or thin-film wave guides. The coupling of direct measurement antibodies with laser excitation will provide the basis for rapid one-step, sensitive, and easy-to-use biosensors for detection of food pathogens. The ability for rapid early detection of *Salmonella* will enhance the safety and quality of U.S. food products.

9902874 Evalution of Jenseniin G as a Potential Food Preservative
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Postdoctoral Fellowship; Grant 99-35201-8265; $90,000; 2 Years

Bacteriocins, proteins produced by some bacteria which kill other bacteria, offer a defense against some foodborne pathogens. Currently, nisin is the only approved bacteriocin for use in foods in the United States. The development of additional bacteriocins for use as food preservatives is needed in the event organisms develop resistance to nisin or for use in foods in which nisin is ineffective. Jenseniin G, a bacteriocin produced by *Propionibacterium jensenii*, inhibits the outgrowth of the causative agent of botulism and is heat and pH stable. These qualities make jenseniin G attractive as a biological food preservative and this proposal addresses its evaluation. Objectives include increasing the number of organisms which are sensitive to jenseniin G, characterizing the mode of action of jenseniin G, and producing large amounts of jenseniin G. Foodborne pathogens will be subjected to conditions which will weaken the bacteria to determine if the compromised cells become more sensitive to the action of jenseniin G. The mechanism by which jenseniin G kills other bacteria will be determined and compared to that of nisin, a known pore-forming bacteriocin. If the mode of action of jenseniin G is different, then the simultaneous addition of jenseniin G with a pore forming bacteriocin may increase the efficiency of killing above that achieved by either bacteriocin alone. Large quantities of protein which are difficult to obtain from *Propionibacterium* will be needed if jenseniin G is to be used as a food additive. Three systems will be examined for the over production of jenseniin G.

9902764 Mechanism of Pathogen Survival During Microwave Thermalization
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New Investigator Award; Grant 99-35201-8609; $123,000; 2 Years

Contamination of foods with microorganisms does occur, and cooking by the consumer is one of the last steps in food preparation that will kill microorganisms. Consumers, especially in high-risk groups, increasingly rely on microwave heating to cook or re-heat ready-to-eat foods. It has been reported that microorganisms are more likely to survive in foods cooked using microwaves than foods cooked using conventional methods. This increases the concern for the food safety of microwave cooked foods. Therefore, heating procedures using microwaves that assure product safety should be identified. Recent foodborne disease outbreaks with fresh produce have highlighted the fact that very little research has been conducted on the effectiveness of microwave heating on vegetable products or products that have fresh vegetables as a component. This project will determine the mechanism of microorganism survival during microwave heating; determine the role of fat in the food product on inactivation of the microorganisms; study the effect of the presence or absence of steam on inactivation of

the microorganisms; and develop mathematical models to describe the cooking process in the microwave oven in the presence or absence of steam. Specific products to be tested include ground beef at different fat levels; chicken breasts with and without skin; broccoli; and a ready-to eat meal-containing broccoli. This research will result in procedures that can be used to develop microwave-heating procedures that will increase food safety.

9902873 Molecular Characterization of the *Campylobacter jejuni* Adhesin to Fibronectin

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Grant 99-35201-8579; $195,000; 3 Years

The ultimate goal of the research outlined is to develop intervention and control methods to reduce the number of cases of human campylobacteriosis caused by *Campylobacter jejuni* and *Campylobacter coli*. *C. jejuni* is a leading cause of human gastrointestinal disease worldwide, causing between two to eight million cases of diarrheal illness per year in the United States. Infection with *C. jejuni* is characterized by fever, severe abdominal cramps, and diarrhea containing blood and leukocytes. Most cases of campylobacteriosis are sporadic in nature, resulting from the consumption of *Campylobacter*-contaminated chicken, unpasteurized milk, and unchlorinated water. The proteins that mediate the binding of *C. jejuni* to host cells are termed adhesins. The goal of this project is to further characterize one *C. jejuni* adhesin termed CadF, for *Campylobacter* adhesin to fibronectin. CadF is conserved among *C. jejuni* and *C. coli* isolates and is required for the colonization of newly hatched Leghorn chickens. These data suggest that CadF may be useful as an acellular vaccine candidate. The results of this research will better define the pathogenic mechanisms and virulence determinants of one enteric pathogen, *Campylobacter jejuni*, and will lead to new intervention strategies to reduce the high carriage incidence of *Campylobacter* in poultry and other reservoir hosts.

EPIDEMIOLOGICAL APPROACHES FOR FOOD SAFETY

Panel Manger - Dr. Richard Isaacson, University of Illinois
Program Director - Dr. Mary Torrence

Epidemiological studies are important in identifying the multiple factors involved in food safety and providing the science-based data for policy decisions. The Epidemiological Approaches to Food Safety Program was initiated in FY 99 to provide funds for interdisciplinary research on the epidemiology of foodborne pathogens. Projects focus on the identification of sources and reservoirs of pathogenic organisms and their toxins in food, animal feed, and the environment; determination of the levels of microbial contamination in finished food products; identification of risk factors and possible intervention or management strategies; identification of farm-based solutions that contribute to decreased prevalence of foodborne pathogens; development of outcome measures for the impact of intervention or management strategies on microbial contamination; and identification of potential sites of contamination in the processing, transportation, retail setting, and consumer use of food products.

9904291 Ecologic Assessment of *Salmonella enteritidis* var *Typhimurium* in a Dairy Milk Shed
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University of California; Department of Population Health and Reproduction; Tulare, CA 93274
Grant 99-35212-8562; $600,000; 3 Years

The objective of the research is to specifically identify the diversity of *S. typhimurium* in a geographic community with a high concentration of dairy cattle and describe the medical ecology of the bacteria relative to the diversity. We believe that only a portion of the known *S. typhimurium* is associated with dairy cattle and can be identified in milk or on cull animals and some of these may be associated with food-borne and human diseases. In addition, there are specific inputs, reservoirs, and animal management practices that are responsible for the persistence, spread, and expression of this subset of *S. typhimurium* on California dairies. Specifically, we will monitor changes in *S. typhimurium* isolated from a two county, dairy milk shed in central California (400,000 dairy cattle) and identify the genetic variants of these isolates. We will determine the spatial patterns of *S. typhimurium* variants in the milk shed (that is determine where they move in the milk shed) and assess the pathogen, environment, and management factors associated with persistent dairy herd infection with *S. typhimurium*.

9904252 New Methods for Risk Analysis of Infectious Animal Diseases Affecting Food Safety
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Grant 99-35212-8548; $359,515; 2 Years

Current risk analysis procedures for food-borne diseases are inadequate. The long-term goal of our multi-disciplinary research is to refine existing and develop new methodologies to allow for risk analysis procedures for food-borne diseases that are more efficient but still scientifically-based. We will apply the methods to toxoplasmosis in swine, *Escherichia coli* 0157:H7 in feedlot cattle, and Salmonella enteritidis in shell eggs, but the methods will be broadly applicable to other food-borne diseases.

9904260 Following Resistant *Salmonella* Through the Food Chain: a Molecular Ecology Approach
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Grant 99-35212-8680; $814,564; 3 Years

The emergence of multiple-drug resistant *Salmonella* has made antibiotic resistance an important, health issue in medicine and food safety. Once antibiotics are introduced, antimicrobial resistance quickly develops, spreads, and persists, even when antibiotic usage is discontinued. The current model is that food-borne pathogens obtain most antibiotic resistance from a pre-existing pool of drug resistance genes in resident benign bacteria of healthy animals. To assess how rapidly drug resistance develops and how long it persists in animals colonized with *Salmonella*, we will examine the distribution of drug resistance genes among the microbial fauna present on poultry farms. We will identify by PCR and DNA-DNA hybridization, drug resistance genes common to both *Salmonella* and resident bacteria in chickens. Specific emphasis will be placed on tracking integrons, mobile genetic elements responsible for the diversity and rapid evolution of multiple drug resistant bacteria. A novel "integron capture" plasmid will be introduced to the chicken's microflora and followed during the normal rearing period for broiler chickens. The plasmid will be periodically isolated to examine the drug resistance genes acquired by the plasmid's resident integron during the vector's passage among the microbial community of the chicken's gastrointestinal tract. Experiments will be performed in a high-security level, biocontainment facility. Clear definition of the genetic elements within the chicken normal flora that facilitate genetic exchange and persistence of multiple antibiotic

resistance will permit realistic risk assessment of the role of poultry *Salmonella* as reservoirs of antibiotic resistance and agents in its transmission to other animals and humans.

9904242 Molecular Epidemiology of *Salmonella* Transmission in Swine Production Systems
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Grant 99-35212-8625; $885,294; 3 Years

The goal of the proposed research is to identify critical control points for interventions to reduce *Salmonella* infection in modern swine production facilities. Intensive ecological sampling will be conducted in long term studies of 8 large, multi-site, modern swine production facilities. Each farm will be visited monthly for 14 months. At each visit, samples will be obtained from various suspected biological and environmental reservoirs of Salmonella. This includes fecal samples from a cross-section of age classes of swine and from rodents, birds, and medium-sized mammals, as well as samples of insects, feed, water, and pen floor contents. These samples will be cultured for *Salmonella*. The *Salmonella* isolates will be characterized genetically, using modern molecular biological techniques. Using information on genetic similarity and proximity in location and time of sampling, inferences will be made regarding probable modes of transmission of *Salmonella* to swine. It will be possible to identify likely targets for intervention if (a) particular biological and environmental sources of *Salmonella* are consistently linked (genetically, temporally, and spatially) to swine, (b) during times of low prevalence, *Salmonella* is maintained primarily in one reservoir, or (c) new genotypes entering the farm are consistently identified first in a specific reservoir. This project is relevant to the sustainability of US agriculture in that it addresses the NRI program objectives of identifying sources of pathogenic organisms in the environment and understanding their ecology, and is designed to promote development of strategies to eradicate disease-causing microorganisms.

9904244 Risk Factors for *Salmonella* and *Campylobacter* Infections and Drug Resistance in Dairy Cattle
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Grant 99-35212-8563; $765,447; 3 Years

A three year longitudinal study of 130 dairy herds is proposed to: 1) identify the patterns of occurrence and shedding of *Salmonella* spp. and *Campylobacter jejuni*, and associated risk factors, on dairy farms in the Midwest and Northeast; 2) evaluate the relative sensitivity and specificity of different sampling frequencies for detection of *Salmonella* and *C. jejuni* in fecal and environmental samples; 3) determine susceptibility profiles of *Salmonella* and *C. jejuni* isolates to a number of antimicrobial agents; 4) compare conventional and organic dairy farms in the Midwest and Northeast with respect to: a) shedding, and b) susceptibility and resistance profiles of the two organisms to specific antimicrobial agents; and 5) determine molecular mechanisms involved in reduced susceptibility and/or development of resistance of *Salmonella* and *C. jejuni* isolates to antimicrobial agents. Dairy farms will be selected from Michigan, Minnesota, New York, and Wisconsin. Fecal, water, milk, feed, and environmental samples will be collected and tested for the presence of specific strains of *Salmonella* and *Campylobacter* using standard molecular techniques. Further typing will be done by a modified Taqman procedure for *Campylobacter*, and phage molecular typing of *Salmonella*. *In vitro* susceptibility testing of the strains to a number of antimicrobial agents will be conducted. Risk factors associated with bacterial shedding and the development of resistance to selected antimicrobial agents will be evaluated using multi-variable statistical methods. Results from this study will provide ways to improve pre-harvest pathogen reduction by identifying factors related to shedding and antimicrobial resistance that can be changed by dairy producers.

9904245 Microbial Contamination of Produce: A Field Study in the Lower Rio Grande Valley, TX
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Grant 99-35212-8564; $416,572; 3 Years

In recent years, new and emerging foodborne pathogens have been described and changes in food production have led to new food safety concerns. Foodborne diseases have been associated with many different foods, including recent outbreaks linked to contaminated fresh fruits and vegetables. This study will examine how specific environmental conditions and farming and produce processing practices are associated with fecal contamination of selected produce groups. Some growing, handling and processing methods used in the produce industry may increase the risk that these foods will become contaminated. The study will describe farming and shipping practices on approximately 12 farms in the lower Rio Grande Valley of Texas with three vulnerable produce groups that are minimally processed and eaten raw (leaf lettuce/spinach, parsley/cilantro/basil, green onions). Key agricultural practices where contamination may occur will be identified by measuring the microbial quality of produce at each step from planting to harvesting, processing and shipping. Sources of fecal contamination will be determined by periodically measuring microbial quality of irrigation and process water, measuring fecal indicator organisms on hand rinses from farm laborers and handlers, and conducting sanitary surveys of

sources of human and animal feces in and around the farms and processing areas. Information gained through this study will be used to formulate recommendations for the produce industry to focus resources on the most critical risk factors for microbial contamination of produce and design effective intervention measures to prevent contamination and foodborne disease.

9904236 Ecology of Antimicrobial Resistance of Enteric *Salmonella* and *E. coli* in Cattle Operations

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Grant 99-35212-8589; $771,868; 3 Years

Antimicrobial drugs are commonly used in food animal production for the treatment of clinical disease and for the enhancement of animal performance. Recently, the use of antimicrobial drugs in food animals has been implicated as an important cause for the emergence of antimicrobial-resistant bacteria of public health concern. However, there is little more than circumstantial evidence that this use of antimicrobial drugs poses a serious public health risk. In particular, there is little evidence upon which to base changes in food animal production practices which might reduce any potential public health risk. Our goal is to describe the on-farm ecology of *Salmonella* spp. and generic *E. coli* antimicrobial resistance patterns in intensively managed cattle operations. We have chosen this goal because beef products are an important vehicle for the food-borne transmission of bacteria. *Salmonella* and generic *E. coli* represent an important food-borne pathogen and a normal inhabitant of the bovine intestinal tract, respectively. Our objectives are to describe the antimicrobial resistance patterns of *Salmonella* spp. and generic *E. coli* recovered from intensively managed cattle operations, and to identify patterns of antimicrobial use and other factors which promote the development of antimicrobial resistance of bacteria in these operations. We will accomplish these objectives using intensive monitoring of dairy and feedlot cattle populations. By accomplishing these objectives, we expect to obtain detailed information which will help to fill the void of information regarding the development of antimicrobial-resistant bacteria in cattle operations, and lead to the development of practical intervention programs.

9904289 Dynamics of *Campylobacter* Transmission on Poultry Farms

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Grant 99-35212-8517; $384,284; 3 Years

Campylobacter jejuni has emerged as the leading bacterial cause of food-borne human enteritis. Many studies have revealed that poultry products are the major source of human exposure to the pathogen. The high prevalence of this pathogen on poultry farms, especially in broiler chickens, provides a strong rationale for reducing the pathogen level at the preharvest stage. Although many farm-based epidemiological studies have been conducted, the manner in which *C. jejuni* enters a flock and how it is maintained within a flock are poorly understood. Lack of this information has hampered the design of effective intervention strategies on farms. The major goals of this multidisciplinary project are to elucidate the sources of *C. jejuni* infection in broiler chickens and to examine the host and environmental factors that affect the transmission of the organism on broiler farms. Our hypothesis is that transmission of the organism is likely a complex and dynamic process, which is mediated by both vertical and horizontal transmission and affected by the host's immune status and the environmental conditions in the production system. The specific aims of this project are to conduct both laboratory and field studies: i) to assess the effect of host immune status on horizontal spread of the organism in young chicks; ii) to determine if vertical transmission of *C. jejuni* occurs from breeders to progenies; and iii) to identify the potential sources and routes for horizontal spread of *C. jejuni* on poultry farms. These studies address key issues in transmission of *C. jejuni* and should yield information useful for controlling this important foodborne pathogen.

9904281 Effect of Water Chlorination on Prevalence of *Escherichia coli* O157:H7 and *Campylobacter* in Feedlot Cattle

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Grant 99-35212-8561; $325,528; 2 Years

Cattle slaughtered while infected with bacteria such as *E. coli* O157:H7 or *Campylobacter* spp. are a probable source of these bacteria in contaminated beef products, and eventually in humans consuming these food products. Our preliminary data leads us to believe that infection of cattle with these agents, like human infections, most often follows their eating contaminated feeds or drinking contaminated water. *E. coli* O157:H7 can be frequently isolated from cattle water troughs, and has been shown to persist in troughs for months, so water is a logical starting point for control of these infections. Our research question is: "Does providing cattle with a cleaner water supply reduce the number of cattle infected with *E. coli* O157:H7 and *Campylobacter*?" We will chlorinate the water supplies of 32 pens of feedlot cattle in two feedlots through the feeding period and compare the infection rate in these cattle to that of cattle consuming non-chlorinated water. Also, we will evaluate the effect of chlorination on water consumption and weight gains. If chlorination of cattle water

reduces cattle infections with bacteria that cause human disease, but is neutral or beneficial on cattle welfare and weight gain, this will be the first proven management practice enabling beef producers to reduce the risk of human disease due to these food-borne enteropathogenic bacteria.

ANIMALS

Research across a broad range of animal systems areas is urgently needed for the future enhancement of animal production efficiency, as well as to address such areas as the modification of animal products. To accomplish this, grants are awarded in four broad areas of research: (1) animal reproductive biology, (2) cellular growth, development and nutrient utilization of animals, (3) animal genome and genetic mechanisms, and (4) animal health and well-being. Emphasis is given to innovative approaches to research questions related to animals primarily raised for food or fiber. This includes aquaculture species and those animals such as horses that contribute significantly to the agricultural enterprise of the country.

ANIMAL REPRODUCTIVE EFFICIENCY

Panel Manager - Dr. William J. Murdoch, University of Wyoming
Program Director - Dr. Debora L. Hamernik

The primary objective of this program area is to increase our knowledge of reproductive biology in agriculturally important animals with the goal of increasing reproductive efficiency. This program supports innovative research on: (1) factors controlling ovarian function including follicular development, ovulation, and corpus luteum formation and function, (2) factors controlling male reproduction, (3) gamete physiology, including oogenesis and spermatogenesis, gamete maturation, and mechanisms regulating gamete survival *in vivo* or *in vitro*, (4) mechanisms affecting embryo development and survival, mechanisms of embryo-maternal interactions, embryo-implantation, development of optimal culture methods for embryos, and causes and remediation of early embryonic loss, (5) mechanisms involved in placental function, and (6) parturition, postpartum interval to conception, and neonatal survival.

Because alterations in animal behavior and animal well-being may impair fecundity, this program also encourages research on the mechanisms controlling animal responses to physical and biological stresses that impinge upon reproductive processes. Research should contribute to an understanding of the causes, consequences, and avoidance of stress, rather than merely describing the physiological effects of stress on reproductive efficiency.

9902240 Sperm Interaction with the Homologous Oocyte Plasma Membrane
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Grant 99-35203-7659; $210,000; 2 Years

The long-term goal of this research is to further our understanding of fertilization. Such an understanding is necessary to improve the reproductive efficiency of food-producing animals. Since a large proportion of the costs of pork and beef production is the cost of maintaining the breeding herds, increasing reproductive efficiency can lower overall costs. The ability of pig and sheep sperm to interact with the oocyte plasma membrane appears to be highly predictive of relative fertility of different ejaculates. Reduced interactions between the sperm and the oocyte plasma membrane may contribute significantly to fertilization inefficiencies in these species. Increased understanding of sperm-oocyte interaction should also increase the production efficiency for high quality *in vitro* fertilized embryos for biotechnological applications. Our specific objectives are to continue characterization of the pig sperm and oocyte plasma membrane proteins that may interact with each other during fertilization and to evaluate ejaculates to determine the distribution of such molecules in sperm cell populations. Techniques to accomplish the first main objective rely heavily on current cDNA protocols. Procedures to determine distribution of interacting molecules on individual sperm cells within an ejaculate rely on specific antibodies to extracellular portions of the interacting molecules and flow cytometry techniques. This may help answer the question of what makes one sperm more fertile than another. In addition to increased understanding of the fertilization process which should lead to long-term improvements in reproductive efficiency, results may lead to a practical assay of fertilizing ability and improvements in reproductive efficiency.

9902219 Photorefractoriness in Turkeys: A Neural Locus and its Neurochemical Anatomy
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Grant 99-35203-7660; $172,000; 3 Years

Turkey breeder hens are routinely induced to lay eggs by exposing them to long daylengths. Turkey poults hatched from these eggs grow to become market turkeys for meat consumption. Long daylengths stimulate turkey hens to lay 100+ eggs per reproductive season, but this number is well below the 300+ eggs laid by chickens. A major reason underlying this difference is that turkeys become refractory to long daylength stimulation ("photorefractory"), whereas chickens do not. This proposal uses a novel approach, induction of the immediate early gene product, c-fos, to identify neurons involved in the transduction of long daylength cues, a response that disappears in photorefractory turkeys. Understanding the neural mechanism of photorefractoriness and identifying the neuronal pathways leading from the perception of long daylengths to the neuronal systems responsible for gonadal growth and egg laying, i.e., those neurons containing the neuropeptide gonadotropin-releasing hormone I (cGnRH I), could lead to the development of technologies to prevent photorefractoriness and increase egg production and reproductive efficiency. The proposal employs immunohistochemistry and *in situ* hybridization to establish the neurochemical identity of neurons that express c-fos as well as nerve axons that contact them. Similarly, the proposal will establish the identity of axons impinging on cGnRH I neurons. We will also determine whether c-fos reactive neurons project to the median eminence to directly regulate pituitary hormone secretion. Determining the neuronal circuitry controlling photostimulation and photorefractoriness is prerequisite to a full understanding of the physiologic mechanisms limiting egg production in photorefractory species.

9902267 Transcriptional Regulation of Placental Hormones

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Grant 99-35203-7817; $270,000; 3 Years

Sub-optimal fetal growth not only impacts the outcome of a given pregnancy, but also impacts postnatal growth and development. If a fetus develops slowly, it will exhibit a light birth weight, compromised neonatal survivability and postnatal growth rate. Conversely, if a fetus grows too fast, it will exhibit excess birth weight, leading to obstetrical complications, reduced neonatal survivability, and often a stunted postnatal growth rate. Regardless of the type of sub-optimal fetal growth, the placenta is the major determinant of the rate of fetal growth through its role in nutrient transfer and the production of placental hormones. One placental hormone, placental lactogen (PL), has been implicated as having a regulatory and/or modulatory role on fetal growth and metabolism. We have isolated and characterized the gene encoding ovine PL (oPL), and have identified regions of DNA preceding the structural oPL gene that likely regulate this gene in a cell-specific fashion. It is our objective to further define the responsible DNA domains as well as the regulatory proteins that interact with these domains. Furthermore, we will examine if these domains can drive placenta cell-specific expression of a reporter gene within transgenic sheep pregnancies. Defining the regions of DNA and regulatory proteins that drive oPL production only in the placenta should provide insight into developing methods for modifying the production of this placental hormone to the advantage of mother and fetus. Furthermore, these regions of DNA could also be used to drive expression of desired products specifically within the placenta.

9902389 Initiation of Folliculogenesis in Sheep

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Grant 99-35203-7719; $180,000; 2 Years

Present methods of improving reproductive efficiency in domestic livestock animals by increasing the number of eggs (oocytes) available for fertilization yield inconsistent results. In sheep and cattle, the majority of eggs are contained in immature (preantral) follicles that have not yet begun to grow. This population of immature oocytes/follicles (86,000/ovary in sheep and 130,000/ovary in heifers) represents an untapped resource for obtaining female gametes. However, a major problem preventing the development of practical methods to utilize this large reserve of immature oocytes is the lack of information regarding signals and mechanisms responsible for initiating follicular growth. To solve this problem research will focus on a newly discovered intraovarian growth factor, growth/differentiation factor-9 (GDF-9), which has been implicated as a critical factor necessary for the initiation of follicular growth, and hence the growth and maturation of oocytes. This novel growth factor appears to be expressed exclusively by oocytes in the ovaries of females. Because of the potential fundamental role of GDF-9 in the process of initiation of folliculogenesis, experiments will be performed to determine if: 1) GDF-9 is expressed in oocytes present in immature follicles cultured *in vitro*, and 2) the functional status (healthy vs atretic) of antral follicles *in vivo* is correlated to the expression of GDF-9. In addition, experiments will be conducted to produce recombinant ovine GDF-9 protein that can then be used for physiological studies. Collectively, the data generated should provide new information critical for the development of practical methods for increasing the number of eggs available for fertilization in livestock species of major economic importance.

9902225 Bovine Somatotropin-Mediated Effects to Increase Embryonic Survival in Cattle

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New Investigator Award; Grant 99-35203-7676; $140,000; 2 Years

A major cause of lowered calf crop and increased calving intervals in dairy operations is suboptimal embryo survival. Bovine somatotropin (bST) increases the rate of early embryonic development in cattle. Goals of this project are to examine the physiological windows and the mechanisms by which bST affects fertility responses in lactating dairy cattle. Our hypothesis is that pregnancy losses often occur because of the failure of the uterus to provide an environment that is conducive to optimal embryonic development and that a portion of these embryos can be rescued by supplemental bST administration to increase availability of insulin-like growth factors (IGFs) that are essential for early embryogenesis. The first objective is to evaluate the stages of pregnancy when embryonic development and survival are most affected by exogenous bST. The second objective is to characterize bST-induced changes in oviductal, uterine and embryonic synthesis of IGFs and their binding proteins (IGFBPs) and to examine whether these biosynthetic activities can be correlated with IGF and IGFBP contents of oviductal and uterine fluids. The third objective is to determine how bST stimulates embryonic development using an *in vitro* model. The fourth objective is to examine the effect of supplemental bST on uterine synthesis of prostaglandin F2 alpha which causes luteal regression in cattle. Knowledge gleaned from this research will provide new strategies to improve embryo survival and pregnancy rates in lactating dairy cows in which fertility is reduced due to high metabolic demands of high milk production.

9902277 20th Annual Meeting of the American Society for Reproductive Immunology
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Grant 99-35203-7689; $10,029; 1 Year

Study of interactions between the immune and reproductive systems can have practical implications for animal production by reducing the incidence of bacterial and viral infections of the reproductive tract, increasing fertility and developing methods for regulation of placental growth and hormone secretion. For this project, the USDA NRICGP is supporting the effort by the American Society for Reproductive Immunology (ASRI) to foster the continued development of large animal reproductive immunology by providing speaker travel for the ASRI meeting to be held in June, 2000. In particular, funds are requested to support five of the speakers whose research involves large animal models. The meeting itself will be organized around five plenary sessions. Plenary Session I, entitled "New Concepts in Reproductive Immunology", will be formatted to highlight some of the recent discoveries that have changed our understanding of mechanisms allowing the fetal allograft to elude the maternal immune system. Plenary Session II will focus on cellular immune function in the reproductive tract, Plenary Session III will be devoted to reproductive microbiology and virology and Plenary Session IV will focus on the regulatory molecules involved in the dialogue between the immune and reproductive systems. The last session (Plenary Session V) is entitled "Nutritional Immunology and Reproduction" and will consist of a series of cutting-edge presentations on an emerging idea that nutritional regulation of immune function can have an impact on reproductive function.

9902282 Influence of Paired Testicular Diameter and Age on Sperm Production in the Boar
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New Investigator Award; Grant 99-35203-7646; $29,692; 1 Year

Previous studies in the boar have established that testicular weight correlates well with daily sperm production, and that testis size is a highly heritable trait. The boar testes are tightly positioned so that it is impossible to obtain circumferential measurement. Consequently, unlike the bull and ram, use of testis size when selecting animals as breeding prospects does not occur in swine. Real-time ultrasonography (RTU) offers science a non-invasive technique for visualizing and measuring tissues in the live animal, including the testes. Application of this existing technology to the boar would bridge the gap between science and current swine production practices by providing the industry with the means to select for potential high sperm- producing boars for breeding programs based on testis size. Experiments in my laboratory have found that RTU is an easy and repeatable technique for measuring testicular diameter. The proposed experiments will ascertain the feasibility and accuracy of RTU testis measurement in relation to sperm production and boar age. The study will use proven, hybrid boars standing as commercial US studs. Boars will be grouped by age, with RTU measurements of both left and right testis diameters obtained. Paired testis diameters will then be compared to a total sperm number composite which will be calculated from the boar's four previous semen collections. If results are favorable, the impact of this study on the swine industry is anticipated to be immediate and far reaching in how boars will be selected for AI breeding programs.

9902675 Gamete Adhesion Molecules in Swine
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Grant 99-35203-7677; $195,000; 3 Years

Fertilization failure and spontaneous early death of embryos in the uterus account for major economic losses in animal agriculture. However, we have a very poor understanding of how sperm and eggs interact. Our knowledge of the key proteins that allow sperm to bind eggs is particulary deficient in agricultural animals. The purpose of this proposal is to identify the genes encoding proteins on porcine sperm that are important to bind sperm to eggs. One or more of these sperm proteins will also help sperm release enzymes so sperm can penetrate through the egg coat and fertilize the egg. We will determine the region of sperm that binds the egg coat. We will use a screen to identify proteins that bind the egg coat and we will be certain they are found on sperm. We will test the biological importance of these proteins during fertilization by blocking them. A better fundamental understanding of fertilization is essential to improve fertilization and early development success rates. One may be able to use these results to develop more accurate fertility assays or add supplements to semen from subfertile males to improve their fertility. These studies will identify and study sperm proteins that bind the egg coat on swine, a species with great agricultural importance.

9902244 Zebrafish Mutagenesis: Isolation and Characterization of Reproductive Mutants

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Grant 99-35203-7718; $210,000; 2 Years

The mechanism and regulation of sperm and egg production by the testis and ovary is complex. Research on these processes has provided valuable information but has been restricted by the experimental approaches employed. In many cases, past studies have concentrated on mediators initially identified from other physiological systems. The present study uses the zebrafish as a general vertebrate model since a great deal is known about the genetics of this species, and because of its size and breeding characteristics, large numbers can be efficiently maintained and bred in animal facilities. The zebrafish is being used by many researchers as a model for animal development. In the present study, an experimental approach is used in which there is no preconceived idea of what is important for the control of testicular and ovarian function. The genes of zebrafish are randomly mutated and progeny with specific mutations in the structure and/or function of the testes and the ovaries are then isolated. Specific testicular and ovarian mutations will be further characterized by microscopy and the genes that have been mutated will be localized to specific chromosomes. The ultimate objective of this program is to identify and characterize the specific genes that have been mutated and are responsible for the reproductive mutations observed. From this, pivotal genes involved in the control of the testis and ovary can be uncovered. This information can be used in the future for the artificial control of reproduction or to understand and circumvent reproductive dysfunctions in commercially important agricultural animals.

9902206 CAR1, A Novel Avian Receptor Mediating Granulosa Cell Apoptosis

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Grant 99-35203-7736; $235,000; 3 Years

During the reproductive life span of most higher vertebrate females (including domesticated mammals and birds), the vast majority of ovarian follicles (80-99%, depending upon species) are normally lost via a process called follicle atresia. However, an abnormal, excessive depletion of the ovarian follicle pool through atresia plays a critical role in causing sporadic and/or infertile reproductive cycles, and results in the loss of reproductive productivity in domestic animals. It is now widely accepted that the loss of ovarian follicles is due to a specific type of cell death (apoptosis) in a select cell type (the granulosa cell) within the follicle. Nevertheless, the identity of signals (ligands and receptors) and their mechanisms of action leading to apoptotic cell death remain largely undefined. Recently a novel cell membrane receptor, called CAR1, has been identified in the chicken and turkey. We have determined that the CAR1 receptor is highly expressed in hen ovarian granulosa cells, and that activation of this receptor induces apoptosis in cultured granulosa cells. We hypothesize that the novel CAR1 receptor is an important physiological regulator of granulosa cell apoptosis, and thus follicle atresia. Knowledge of this novel receptor and its intracellular mediators of apoptotic cell death in granulosa cells has clear implications for understanding basic processes related to follicle atresia as well as to normal follicle growth and development, and by implication, its potential relationship to subfertility and infertility in domestic animals.

9903453 Intracellular Signals Regulating Follicle Growth

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Grant 99-35203-7735; $190,000; 3 Years

Innovative research is needed to provide fundamental information on the intracellular signaling mechanisms which control follicle growth and development. It is well established that insulin-like growth factor-I (IGF-I) and epidermal growth factor (EGF) are important regulators of granulosa cell proliferation and development of the pig follicle. The long-term goal of this project is to determine the intracellular signals utilized by growth factors that allow granulosa cells to survive and proliferate in order to successfully support the ovum to a stage leading to ovulation. These studies will provide new mechanistic information on the cellular events which control the survival and proliferation of ovarian cells and are expected to translate into more effective strategies for enhancing reproductive efficiency. In this proposal, we will test the overall hypothesis that IGF-I-induced signals are required for granulosa cell survival and amplification of EGF-stimulated granulosa cell proliferation. Our preliminary data show that each of these important growth factors can activate multiple intracellular signaling systems in granulosa cells. These signaling pathways regulate events that control gene transcription or the activity of proteins that direct the fate of the cell. These results provide an important experimental model which we now propose to use to address the intriguing question of which signaling pathways are responsible for protecting granulosa cells from cell death and/or promoting cell proliferation.

9902371 Isolation and Identification of the Boar Sperm Ca2+ Oscillogen

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Grant 99-35203-7688; $200,000; 3 Years

The sperm, during mammalian fertilization, induces a series of intracellular calcium rises that are required for oocyte activation and embryo development. The mechanism(s) by which the sperm generates these calcium responses remains unknown. Our preliminary information, using sperm cytosolic fractions (SF), shows that injection of SF into mammalian oocytes evokes persistent intracellular calcium oscillations similar to those observed during fertilization. We hypothesize that mammalian sperm contain a cytosolic calcium-release activating compound(s) and our preliminary observations indicate that it can be isolated/identified by using a combination of fractionation techniques. We will use conventional chromatographic approaches to generate active fractions with limited polypeptide composition, coupled with identification of these polypeptides by modern sequencing technologies and immunological (immunodepletion) techniques. Identification of this molecule is important. First, it would identify the molecule(s) triggering mammalian development. Second, from an applied side, it would provide a method to improve and optimize parthenogenetic activation of mammalian oocytes. This would be significant to use cloning effectively and intracytoplasmic sperm injection (ICSI) techniques in several species of economical importance in which activation remains a significant problem. Finally, this molecule(s) is likely to be conserved across species and, once identified and cloned, it could be used as part of a comprehensive male fertility screen. In summary, identification of this molecule(s) may offer insights into the regulation of calcium release in oocytes and in other systems, and may also have immediate practical applications in domestic animal cloning techniques and assisted reproductive technologies.

9902385 Epidermal Growth Factor Receptor Activation in Bovine Oocyte Development

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Grant 99-35203-8679; $165,000; 3 Years

This project aims to understand the function of a natural protein known to enhance the growth of cow oocytes. This protein, epidermal growth factor (EGF), acts in many cell types by binding with another cellular protein, the EGF receptor, and thereby activating the EGF receptor. This activation leads to numerous changes within cells, ultimately causing them to progress through cell division and growth. Cow oocytes surrounded by their closely associated cumulus cells are known to develop more robustly if EGF is added to the fluid in which they are bathed. The mechanism by which this enhancement of development occurs is not known. This project will study changes in several aspects of oocyte and cumulus cell function in response to EGF stimulus. Among the aspects of cell function to be studied are ion movements within individual cells, ion flux from complexes of cells, movements of biomolecules between cells, redistribution of relevant biomolecules within cells, and the effect of introduction of a permanently activated EGF receptor. This project will also examine the mechanism behind and role of cell death that appears to be activated in mammalian oocyte-cumulus cell complexes. Because the proper development of cow oocytes is of major importance to the dairy and beef industries, this work has relevance to US agriculture. Results from this study may allow these industries to improve the reproductive efficiency of beef and dairy cattle.

9902239 Calcium Entry and Extrusion Mechanisms in Porcine Oocytes

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University of Missouri, Columbia; Department of Animal Sciences; Columbia, MO 65011-5300
Grant 99-35203-7675; $183,000; 2.5 Years

The objective of the project is to investigate the mechanism of calcium signaling in pig eggs. In sexual reproduction, the sperm and the egg unite in the process of fertilization before development of a new organism can begin. The fertilizing sperm activates the egg by inducing a transient increase in the egg's free calcium concentration that in mammals is followed by a long-lasting calcium oscillation. Although the oscillation is very important, because it gives the signal for the onset of development, its exact mechanism is still not clear. It is generally accepted that the calcium comes from calcium stores inside the egg. Since prolonged high calcium levels are detrimental to the cell, calcium has to be pumped back into the stores or extruded across the plasma membrane. External calcium is also thought to enter the egg and help replete the empty stores. It seems that it is the regulated uptake and re-discharge of calcium by the stores that generate the oscillation which is maintained by the synchronized operation of the calcium entry and extrusion mechanisms located in the cell membrane. However, the machinery that is involved in the translocation of calcium across the cell membrane is not known. Understanding this machinery and its function will help in improving methods of artificial oocyte activation, which has growing importance not only in the field of *in vitro* fertilization and assisted reproductive technologies but also in embryo cloning and biomedical research.

9902245 Bull-cow Interactions Attenuate the Cow-calf Bond Regulating Anestrus
Berardinelli, J.G.
Montana State University; Department of Animal and Range Sciences; Bozeman, MT 59717-2900
Strengthening Award; Grant 99-35203-7932; $95,000; 3 Years

The goal of this research is to determine how the presence of bulls reduces the period of non-reproductive activity of cows nursing calves. We will evaluate the novel hypothesis that bulls and cows form a "bonded" relationship that either reduces the negative effect of nursing calves (the cow-calf bond), or directly stimulates the brain centers responsible for initiating the onset of reproductive activity. In these experiments we will determine if the presence of bulls decreases the cow-calf bond allowing establishment of a bull-cow "bond": accelerating early return to reproductive activity. We will then determine whether the bull produces pheromones (chemical agents produced by individuals that alter the behavior of others of the same species) that in and of themselves are sufficient to "break" the cow-calf bond or require the behavioral aspects of bulls to do so. Lastly we will attempt to show that a cow-bull bond is established when calves become more "independent" of dams, when the bull effect becomes evident in cows, by substituting an "alien" bull for a "familiar" bull (exposed to cows soon after calving). Results of these studies should support the novel idea that bulls reduce the postpartum non-reproductive period by forming a social or behavioral bond with cows, when calves become less dependent upon dams. If this idea proves to be correct, it could substantially improve our understanding of the important role of bulls in the reproductive activity of cows. With such understanding, new cost-effective management strategies may be developed and tested that employ bulls to enhance the reproductive efficiency and sustainability of beef cattle production in the United States.

9902220 Increasing Ovulation and Spermatogenesis with an Inducible FSH-beta Transgene
Miller, W.L.
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Grant 99-35203-7661; $200,000; 3 Years

Follicle-stimulating hormone (FSH) is produced in specialized cells (gonadotropes) found in the pituitary and it has major control over reproduction (egg and sperm production). Many have tried to manipulate endocrine hormones to increase FSH so that birthing rates of domestic animals might be increased, but all efforts to date have failed. Proposed here is a plan to insert into animals an extra FSH gene that can be switched on at times critical for reproduction. This gene will be tested in mice (transgenic mice) to determine if the approach will be useful in domestic livestock. The first step is to express the FSH-beta gene specifically in pituitary gonadotropes using the promoter of the beta subunit of sheep FSH. A second step is to express the tetracycline receptor along with the FSH gene, a procedure that has recently been refined by USDA researchers. Three aims are outlined below that will lead to the production of transgenic mice which will carry the tetracycline-inducible FSH gene and the tetracycline receptor exclusively in pituitary gonadotropes. The ability of a tetracycline derivative, fed to the mice, to increase FSH production, egg and sperm production, and birthing rates will then be tested. To accomplish this overall goal we propose in AIM 1 to link the sheep FSH beta promoter and cow FSH-beta gene together in the USDA-designed tetracycline-sensitive DNA plasmid and test FSH synthesis in cultured cells. AIM 2 is to produce transgenic mice carrying the FSH transgene and determine if the FSH transgene can be activated by a tetracycline analogue to increase birthing rates. AIM 3 is to characterize changes in the ovaries and/or testis in transgenic mice when FSH is increased by feeding a tetracycline derivative. Successful completion of this proposal will lay the foundation for using high production of FSH to increase fertility and birthing rates in domestic and/or exotic animals. This goal is important given the current pressure for reducing breeding stock for environmental reasons. The study will also provide basic information on the long-term effects of controlled secretion of FSH on ovarian and testicular function.

9902381 The Role of Relaxin in Porcine Uterine Growth and Remodeling
Bagnell, C.A.
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Grant 99-35203-7812; $180,000; 3 Years

Stimulation of uterine growth during pregnancy is critical to accommodate growing fetuses and ensure embryo survival. Embryonic mortality during early pregnancy is a major source of reproductive loss in farm species, particularly in the pig. Improving embryo survival in pigs is key to increasing reproductive efficiency, producer profitability and ultimately the world food supply. Relaxin, a hormone of pregnancy, increases growth of reproductive tissues in pigs. However, the means by which relaxin promotes growth is not well understood. The working hypothesis is that relaxin acts as a uterine growth factor by enhancing remodeling and cell communication. This hypothesis will be tested *in vivo* in a prepubertal pig model in which the uterotrophic effects of relaxin have been documented, and *in vitro* using a porcine endometrial cell culture system. In objective 1 the ability of relaxin to increase uterine growth by altering the balance between the matrix metalloproteinases (MMP) and their inhibitors, the tissue inhibitors of MMPs will be tested. Objective 2 will focus on the impact of relaxin on uterine cell-cell communication through gap junctions and vascularization by expression of vascular endothelial growth factor. In objective 3 whether the uterotrophic actions of relaxin are mediated via ligand-independent

activation of the estrogen receptor will be investigated. Results from these studies will provide new information concerning the means by which relaxin promotes uterine growth. This information could lead to the development of innovative strategies to regulate reproductive tissue growth, improve embryo survival and thereby enhance reproduction in food animals.

9902212 Paternal Contributions to the Functional Zygotic Structure in Bovine

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New Investigator Award; Grant 99-35203-7785; $215,000; 3 Years

Mitochondria, the cell's energy-producing factories, carry a small number of genes, which similar to the genes on the cell's chromosomes, encode vital genetic information. The sperm mitochondria and their genes suffer substantial damage during their passage through the female genital tract and are therefore destroyed inside the egg after fertilization. The egg's own mitochondria, in contrast, are kept intact. Although the destruction of sperm mitochondria after fertilization is believed to prevent the perpetuation of damaged, potentially deadly paternal mitochondrial genes, the actual mechanism of this event is not known. Moreover, such a mechanism is not in place when assisted reproductive technologies are used to produce transgenic cattle. Preventing the perpetuation of harmful mitochondrial genes from donor cells will be necessary for successfully using cloning technology without creating defective animals such as the cloned calves reported recently. This project is based on the finding that the small protein ubiquitin, used as a common marker for the destruction of damaged proteins inside cells, specifically marks bull sperm mitochondria for destruction inside the bovine egg. Three specific aims seek answers to the following questions: 1. When are the sperm mitochondria marked by ubiquitin during their development and after fertilization? 2. What is the cellular machinery involved in the destruction of the sperm mitochondria? 3. Which sperm proteins are recognized and marked by ubiquitin? This knowledge has important implications for U.S. agriculture, as it will elucidate the mechanism by which the unwanted mitochondrial genes can be eliminated during natural and assisted fertilization.

9902256 Regulation of Bovine Sperm Capacitation by Protein Tyrosine Phosphorylation

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Grant 99-35203-7674; $225,000; 2.5 Years

Prior to being able to fertilize an egg, mammalian sperm must undergo a complex, but ill-defined, series of events termed capacitation. While capacitation is absolutely critical for successful fertilization, it is one of the most difficult obstacles to successful *in vitro* fertilization and the molecular basis underlying these events is poorly understood. The long-range objectives of this proposal are directed towards understanding the cellular and molecular mechanisms regulating bovine sperm capacitation. The experimental rationale for this grant is the finding by our group that the phosphorylation of sperm proteins on tyrosine residues in mammalian (mouse, human, and bovine) sperm may represent a common regulatory pathway that modulates events associated with capacitation. In specific aim 1, we will characterize those bovine sperm protein substrates that become tyrosine-phosphorylated during capacitation using a combination of cellular and molecular approaches that will allow us to identify and localize these proteins. In specific aim 2, we will purify and characterize the proteins (called kinases) that are responsible for phosphorylating these substrate proteins during sperm development. Because capacitation is crucial for successful fertilization, increasing our understanding of the molecular events of capacitation will allow us to develop diagnostic tools for assessing this event and to increase its efficiency by controlling it *in vitro*. The biological issues of capacitation are important considering the economic ramifications of improving the reproductive efficiencies in the cattle breeding industry.

9902268 Gordon Research Conference on Fertilization and Activation of Development

Myles, D.G.
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Grant 99-35203-7608; $2,000; 1 Year

The Gordon Conference on Fertilization and the Activation of Development will be held at the Holderness School, Plymouth, New Hampshire, July 25-July 30, 1999. This conference is concerned with the interaction of the male and the female gametes in the vital process of fertilization and activation of the fertilized egg. There will be nine sessions in the conference. The conference provides a venue for the interaction of biochemists, cell biologists, molecular biologists, physiologists, geneticists and biophysicists investigating fertilization and the activation of development. This conference has been singularly successful in fostering collaborations between investigators with widely disparate experience and seniority. In these ways it is of benefit to the advancement of our understanding of fertilization and the activation of the egg that initiates development of the new organism. Understanding of these phenomena is the basis for improvements in animal breeding (including *in vitro* fertilization, intracytoplasmic sperm injection and cloning).

9902281 Origin and Significance of Turkey Seminal Plasma Proteolytic Enzyme(s)

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Strengthening Award; Grant 99-35203-8161; $120,000; 2 Years

The domestic turkey is unique among avian species in that a proteolytic enzyme (TSPE), active at basic pH, is found in seminal plasma. The objectives of this research are to: 1)determine the origin of TSPE within the reproductive tract; 2)biochemically characterize TSPE; and 3)investigate the effects of TSPE or TSPE inhibitors on sperm mobility and fertilizing capacity of stored semen. A monoclonal antibody to TSPE will be produced and used to immunostain sections of the turkey male reproductive tract to determine sites of enzyme production and/or concentration. Enzyme kinetics will be studied using synthetic substrates and proteinase inhibitors. TSPE activity will be determined in pooled turkey semen immediately after collection and periodically during storage at 4C for up to 48 hours. The effects of adding TSPE or proteinase inhibitors to semen during storage will be determined by evaluating sperm mobility. If addition of TSPE or proteinase inhibitors to stored semen results in improved sperm mobility, fertility trials will be conducted to determine if a similar beneficial effect is observed for sperm fertilizing capacity from semen stored for 24 hours. Fertility, hatchability of eggs, and embryonic mortality will be examined. The knowledge generated from this research will add to the understanding of avian semen physiology and may result in improved methods for semen storage. This would benefit the turkey breeding industry, where semen is routinely pooled and stored prior to use for artificial insemination.

9902227 Retinol in Oocyte Maturation and Early Embryonic Development

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Grant 99-35203-7709; $215,000; 3 Years

Vitamin A (retinol) has long been recognized to be essential for embryonic development and survival. In the cells of the body, vitamin A is converted to retinoic acid, which has recently been identified as an important molecule for cell growth and differentiation. Recent results from our laboratory provide convincing evidence that injection of retinol into animals, during the time when eggs within the ovary are undergoing their final maturation, improves the capacity of these eggs to develop into healthy embryos after fertilization. The addition of retinol to embryos produced by *in vitro* fertilization, also improves the capacity for embryos to survive. The long-term goal of this proposal is to improve reproductive efficiency of livestock through retinol treatment. To this end, we have developed a whole animal model (*in vivo* system) and a laboratory cell culture model (*in vitro* system) to investigate the actions of vitamin A. The specific aims are: (1)demonstrate that our novel retinol treatment protocols improve the competence of embryos to survive manipulation, maintain pregnancies and produce healthy offspring; (2)determine the site of action of the retinol treatment (i.e., are positive actions of retinol affecting the egg in the ovarian follicle or the embryo in the oviduct and uterus?); (3)determine the effects of retinol treatment of animals (*in vivo* model system) on egg morphology, energy metabolism and protein synthesis; and (4)determine the *in vitro* effects of retinol, administered during egg maturation, *in vitro* fertilization and culture of *in vitro* produced embryos, on embryonic development, metabolism, protein synthesis and ability to survive manipulation (freezing and transfer). Results from these studies will provide valuable information on the role of retinol in early embryonic development and may provide novel methods to improve reproductive efficiency in livestock.

9902270 Regulation and Function of a Progestin Receptor on Fish Sperm Membranes

Thomas, P.
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Grant 99-35203-7786; $200,000; 3 Years

The proportion of fish eggs that are fertilized during spawning is greatly influenced by the quality of the sperm provided by the males. Sperm motility varies considerably in many important aquaculture species both between males and within the same individual at different stages of the reproductive cycle, but the underlying causes are unknown. The purpose of this project is to obtain a better understanding of the mechanisms by which sperm activation and sperm quality is regulated by a steroid hormone (20 beta-S) and its sperm membrane receptor in a marine fish, Atlantic croaker. It is hypothesized that the sperm membrane steroid receptor is a critical component of the regulatory system for sperm activation. We will investigate whether steroid (20 beta-S) receptor concentrations influence hormonal activation of sperm, the intracellular changes induced by binding of 20 beta-S to its receptor, and their effects on sperm motility and fertilization capability. The effects of various hormones and drugs on sperm hormone receptor concentrations, sperm calcium concentrations and sperm function will be investigated in tissue incubation systems. It is expected that the knowledge obtained with croaker can be broadly applied in the development of effective hormonal treatments to enhance sperm quality in many aquaculture species. Thus, these studies are likely to be of practical and economic importance in optimizing reproduction of cultured fishes in the United States.

9902247 Bovine Oocyte Activation

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Grant 99-35203-7647; $155,000; 2 Years

Intracellular calcium changes (transients and oscillations) at fertilization are pivotal to oocyte activation and subsequent development. There are at least two types of intracellular calcium channels that have been identified and studied in a large number of different cells. These two calcium channels are the IP3 and ryanodine receptor-mediated channels. Several species of oocytes have been studied to identify the specific type of calcium channels that exist and facilitate oocyte activation. Bovine oocytes contain both IP3 and ryanodine receptors and each has the ability to induce intracellular calcium transients and subsequent activation. Because activation is such an important initial step in development and the production of viable embryos, knowledge of the molecular properties of both the IP3 and ryanodine receptors is crucial for further understanding the calcium releasing mechanisms in oocytes not only in the bovine, but also in other mammalian species. In addition to these two intracellular calcium channels, recent data indicate the potential existence of integrin receptors on the surface of the oocyte, which may help mediate calcium transients at fertilization through either the IP3 or ryanodine calcium channels. Experiments outlined in this project will further evaluate important biological questions associated with extracellular (integrin) and intracellular (IP3/ryanodine) receptors of bovine oocytes. These questions address the specific method by which sperm induce intracellular calcium transients, the pathways (s) involved in generating and sustaining these intracellular transients, and the receptor on the oocyte which is involved in fertilization and subsequent activation. Results of this work will provide new information regarding how the fertilizing sperm can initiate embryonic development. This information will be used to increase the efficiency of nuclear transfer and embryo development.

9902384 Annual Meeting of the Society for the Study of Reproduction

Griswold, M.D.
Society for the Study of Reproduction; Madison, WI 53711-2021
Grant 99-35203-7805; $16,000; 1 Year

The 1999 annual meeting of the Society for the Study of Reproduction is organized around the theme of, “Research in Reproduction in the New Millennium”. In the new millennium, issues involving animal and human reproduction will assume a central worldwide importance. Some of these issues include: greater efficiency of livestock reproduction, decreased fertility in humans and animals, preservation of endangered species and wildlife resources and novel approaches to control reproduction. To address these issues, it is essential to advance our fundamental understanding of the underlying processes at the molecular and biological levels. In the new millennium, the importance of ruminant livestock in providing human food will assume more importance. While the entire scientific sessions will be of interest to scientists working in reproduction in food animals, three minisymposia are designed to focus on reproduction in domestic animals: 1)Sexual Differentiation, 2)Cellular & Molecular Regulation of Luteal Formation, Function and Regression, and 3)Science and Applications of Animal Cloning. Participants in this meeting include academic, Federal and industrial scientists, postdoctoral fellows and graduate students. All speakers in the plenary sessions or the minisymposia are encouraged to submit review articles for publication in *Biology of Reproduction*, the leading journal in the reproductive sciences.

9902383 Meitotic Arrest of Bovine Oocytes by Intracellular Acidification and Hypoxia

Bavister, B.D.
University of Wisconsin; Animal Health and Biomedical Sciences; Madison, WI 53706
Grant 99-35203-7749; $160,000; 3 Years

The role of intracellular pH in controlling arrest of egg maturation within the ovarian follicle will be studied in several ways. Eggs released from their follicles into culture media will be acidified using weak acids, or by blocking an enzyme that normally transports acids out of the cells in exchange for external sodium ions, or by preventing acid release by removing external sodium. Alternatively, effects will be tested of reducing oxygen levels down to those expected to occur within the ovarian follicles, which should change the metabolism of the eggs and other follicular cells so that they produce acid internally. One or more of these approaches is expected to demonstrate that acidifying the cell cytoplasm greatly extends the period during which eggs can remain in culture without resuming maturation. It will be necessary to show that eggs held under these conditions are still capable of maturing afterwards and are developmentally competent. To address this issue, eggs will be fertilized and then cultured to determine if they develop into embryos. Representative embryos derived from the arrest treatments will be transferred to cows to determine if they are viable. The objectives of this research are to assess methods that the ovarian follicles may use naturally to prevent egg development, and to see if acidification of egg cytoplasm can be used to prolong the time that eggs can be stored in culture medium. Prolonged storage of eggs without using freezing techniques would be valuable for embryo research and for long-distance transportation of eggs.

9902242 Deviation in Growth Rates Between the Two Largest Follicles in Cattle

Ginther, O.J.
University of Wisconsin, Madison; Animal Health and Biomedical Sciences; Madison, WI 53706

Grant 99-35203-7669; $220,000; 2 years

The ovarian follicles of cattle develop in waves. Each wave gives rise to a dominant follicle that becomes large and subordinate follicles that regress. The dominant follicle that arises from the last wave of the estrous cycle becomes the follicle that ovulates. This proposal is focused on the time when the future dominant and subordinate follicles of a wave begin to deviate in diameter. The time of the expected beginning of deviation is based on reported means for diameter of the largest follicle at the beginning of deviation (8.5 mm). Deviation is initiated and established rapidly. Tests of hypotheses will require that changes in follicle growth or concentrations of FSH (the hormone that stimulates the development of a wave) must be established in less than 8 hours. Experiment 1 will determine whether a depression in FSH occurs when the largest follicle reaches approximately 8.5 mm. Experiment 2 will consider whether the largest follicle produces a factor that suppresses other follicles indirectly through the depression of FSH concentrations or directly from follicle-to-follicle. This will be done by removal of the largest follicle and determining whether or not growth of the second-largest follicle during the first 8 hours is preceded or followed by an increase in circulating FSH concentrations. Experiment 3 will remove follicles of various diameters to test the hypothesis that deviation involves FSH depression specifically by the largest follicle. Focusing on the rapid (less than 8 hours) development of deviation is a novel approach not used by other laboratories. Because of the pivotal role of deviation in follicle development, its elucidation should provide a fundamental basis for development of practical techniques to manipulate ovarian follicles. For example, knowledge of the status of a wave of follicles should be important for designing optimal techniques for superovulation (clinically causing many follicles of the wave to ovulate) for embryo-transfer purposes.

9902390 Influence of Dietary Oils on Reproduction in Primiparous Beef Cattle

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Strengthening Award; Grant 99-35203-8040; $155,000, 3 Years

The largest reproductive loss experienced by beef producers is failure of cows to conceive before the end of the breeding season. Research indicates that dietary oil supplements may improve beef cow reproduction. Therefore, specific objectives of this project are to: 1)Determine if unique fatty acids in oil supplements are responsible for improved postpartum reproduction; 2) Determine how beneficial effects of oil supplements on reproduction are mediated; and 3) Determine if beneficial effects of oil supplements on reproduction could be due to the presence of estrogenic compounds. Successful completion of the project should enable producers to adopt management strategies which improve beef cow productivity.

ANIMAL GROWTH, DEVELOPMENT, AND NUTRIENT UTILIZATION

Panel Manager - Dr. Alan J. Conley, University of California, Davis
Program Director - Dr. Debora L. Hamernik

Research in this program area contributes to our understanding of the biological mechanisms underlying growth and development in agriculturally important animals. Emphasis is placed on innovative approaches in several research areas including but not limited to: cell proliferation and differentiation, genetic mechanisms underlying growth and development, metabolic regulators such as growth factors, synthesis and degradation of protein and lipid at the cellular or tissue level, metabolic and nutritional aspects of growth and development including rumen microfloral development and cellular and molecular aspects of the effect of environmental stress on growth and development. Development of dynamic modeling systems assessing specific quantitative estimates of nutrient requirements is also encouraged.

9903361 Endotoxin Regulation of Growth Hormone Secretion
Sartin, J.L.; Braden, T.D.; Buxton, D.F.
Auburn University; Department of Anatomy, Physiology & Pharmacology; Auburn, AL 36849-5518
Grant 99-35206-8021; $182,000; 2 Years

Diseases produce major alterations in hormones that may in turn disrupt normal developmental patterns of growth and reproduction. One model for the study of disease effects on animals is bacterial endotoxemia. In sheep and humans, endotoxemia produces an increase in growth hormone release and an inhibition of luteinizing hormone release. There is evidence for both a central nervous system and direct pituitary action of endotoxin or specific cytokines (interleukin-1, tumor necrosis factor) to alter hormone release. This study seeks to: (1) determine whether endotoxin-stimulated GH release is through mediation by central production of interleukin-1 (IL-I) and/or tumor necrosis factor (TNF); and (2) determine whether endotoxin stimulates the increase in plasma GH concentrations via IL-1 or TNF at the pituitary or hypothalamus. The results of this study may allow the development of future strategies to minimize the impact of disease on growth and development in farm animals, which should improve animal health and well being as well as animal productivity.

9903277 Production of All-Female Stocks of Pacific White Shrimp
Moss, S.M.
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New Investigator Award/Strengthening Award; Grant 99-35206-8258; $130,103; 2 Years

The objective of this project is to establish methods to produce all-female populations of Pacific white shrimp (*Litopenaeus vannamei*) to increase productivity of U.S. shrimp farms. Female white shrimp are 10-20% larger than males at harvest. Our long-range goals are to develop economically viable methods of producing all-female shrimp for U.S. shrimp farmers. We propose to produce all-female shrimp populations by: 1) application of hormones in the water or feed; 2) manipulation of environmental conditions that may affect sex ratios; and 3)determination of family differences in sex ratios and selective breeding for skewed sex ratios. These approaches may allow for the commercial production of all-female shrimp populations as is typically done in salmonid farming.

The U.S. has an annual trade deficit in shrimp products in excess of $2.5 billion. With commercial shrimp fisheries at maximum sustainable yield, increased domestic production of shrimp will likely come from aquaculture. Currently, shrimp farmers rely on outdoor, earthen ponds situated along the coast. These farms are marginally profitable and there is little room for expansion of the industry. Future expansion of the U.S. shrimp farming industry may be dependent on biosecure production systems built away from the coast with little negative environmental impacts. Production of larger, all-female shrimp will increase the profitability of current shrimp farms, as well as the emerging inland, biosecure production systems. Increasing profits and productivity in these more environmentally friendly systems may allow for future expansion of the U.S. shrimp farming industry to all areas of the country.

9903372 Accelerating Bile Acid Synthesis in Developing Swine
Lewis, D.S.; Beitz, D.C.
Iowa State University; Department of Food Science and Human Nutrition; Ames, IA 50011
Grant 99-35206-8621; $200,000; 2 Years

Pork producers wean piglets at early ages to reduce the incidence of many diseases. Early-weaned piglets, however, have immature digestive processes that decrease the efficiency of nutrient absorption. To maintain robust piglet growth and weight gain, early-weaned piglets are commonly fed dry food containing expensive components, many of which are intended to by-pass the immature digestion processes inherent in young piglets. One limitation in digesting and absorbing nutrients in early-weaned piglets is a limited ability to make bile. Bile is required for the efficient absorption of energy-rich fat and important vitamins, such as vitamin A. The major objectives of this grant are to determine 1) the extent that bile limits absorption of cheaper dietary sources of fat (animal fats), 2) the effect of development of the production of important

components of bile, and 3) the capacity of the piglet to increase bile production at an earlier age than normal. This grant focuses on the enzyme that determines how much bile is produced. This enzyme is called cholesterol 7-alpha hydroxylase. Identifying how this enzyme is controlled during piglet development will provide the physiologic and molecular information that is necessary to develop strategies to increase the production of bile at earlier ages. Increasing bile production in early-weaned piglets is expected to increase the efficiency of digesting and absorbing nutrients, which would lessen the reliance on expensive feeds. Ultimately improving piglet digestive development would improve growth and weight gain in piglets weaned to diets supplemented with cheaper nutrients than currently employed.

9903374 Key Proteins Integrating the Myofibrils within Developing and Adult Muscle Cells
Robson, R.M.
Iowa State University; Muscle Biology Group; Ames, IA 50011-3260
Grant 99-35206-8676; $270,000; 3 Years

The skeletal muscle cells (fibers) of animals are literally packed with long contractile protein threads called myofibrils. The myofibrils are, in turn, attached to each other and integrated into the overall structure (cytoskeleton) of developing and adult muscle cells by other key cytoskeletal crosslinking proteins and structures. The long term goal of this basic project is to understand the properties and biological functions of these specific crosslinking proteins. This project will focus on the specific proteins desmin, paranemin and synemin, which form long, very thin structures called intermediate filaments that crosslink adjacent myofibrils, and on other specific proteins including talin and vinculin, which help link the peripheral layer of cellular myofibrils to the outer cell membrane. Integrated studies, involving biochemical, molecular biological and structural methods, will be done to determine the properties and functions of these key proteins and cytoskeletal structures. The myofibrillar and other cytoskeletal proteins make up over half of the protein in muscle, and are responsible for most of the important nutritional and eating properties of muscle foods. Porcine and poultry muscle will be used in the studies, and will yield scientific knowledge of mechanisms involved in growth and development of agriculturally important animals. The efficient growth and development of muscle cells and the production of high quality food (muscle/meat) from domestic animals are, in turn, major goals consistent with national research priorities for animal agriculture.

9903271 Skeletal Muscle Growth by Regulation of Fibroblast Growth Factor Receptor 1 Gene Expression
DiMario, J.X.
Finch University of Health Sciences/The Chicago Medical School; Department of Cell Biology and Anatomy; North Chicago, IL 60064-3095
New Investigator Award; Grant 99-35206-7987; $182,000; 2 Years

Consumption of chicken in the United States accounts for a significant amount of total meat. From 1980 to 1997, chicken consumption per capita increased by 56.6%. In comparison, total meat consumption over the same period increased only 6.9%. The research funded by this grant focuses on a critical factor in the development and growth of the chicken musculoskeletal system. Skeletal muscle cells grow in response to the presence of growth factor proteins – particularly fibroblast growth factors (FGFs). These factors bind to cell surface receptors (FGFRs) which transmit signals into the cell for continued cell proliferation. Therefore, both the growth factor and its receptor are necessary for muscle cell growth. Conversely, a decrease in growth factor and/or its receptor is required for muscle cell differentiation and formation of normal muscle tissue. Our research has shown that the receptor for fibroblast growth factor is down-regulated during chicken skeletal muscle differentiation. To understand the mechanism of this important process in myogenesis, we have isolated the FGFR1 gene and the associated DNA that regulates its expression. The goal of our research is to understand how this gene is regulated. This will be done by identifying and characterizing the DNA elements that are involved in FGFR1 gene expression in both proliferating and differentiating muscle cells. Also, we will determine which protein factors interact with these DNA elements to regulate FGFR1 gene expression. This research will provide important information concerning how muscle cells grow to form normal muscle tissue as well as contribute to our basic understanding of cell proliferation and differentiation.

9903269 Hypothalamic Neurochemical Responses to Leptin
Beverly, J.L.
University of Illinois, Urbana-Champaign; Department of Animal Sciences; Urbana, IL 61801
Grant 99-35206-7985; $182,000; 2 Years

Hormonal, metabolic and behavioral systems regulating growth, development and nutrient utilization of an animal are regulated by neurochemical events in the hypothalamus. How these neurochemical mechanisms influence tissue nutrient utilization and composition are poorly understood. The objective of the proposed research is to define the neural mechanisms coordinating feed intake, energy expenditure, and nutrient utilization to regulate composition of growth in domestic animals. A critical component for regulation of growth is the presence of a signal, or signals, reflecting the current state of energy

balance and body composition. The obesity protein leptin is an endogenous factor affecting feed intake and metabolic rate of animals. Leptin is synthesized by cells in white adipose depots and released into the circulation at concentrations positively correlated with degree of adiposity. Circulating leptin is taken up into the hypothalamus where it acts on receptors to alter behavior, metabolism, and endocrine status. Thus, leptin serves as a signal to the central nervous system conveying the status of peripheral energy reserves. Leptin has been found to affect energy homeostasis in all animals examined with deficits in the protein, or it's receptors, having pathological consequences. Exogenous leptin decreases feed intake, increases energy expenditure, and influences reproductive, thyroid, and adrenal hormones as well as the growth hormone axis. By coordinating the activity of these various systems, exogenous leptin elicits dramatic and rapid changes in energy balance. Knowledge of the neurochemical events mediated by leptin will improve our understanding of the regulation of energy homeostasis and aid in the development of strategies to improve animal productivity.

9903370 Molecular Analysis of Ammonia Assimilation by *Ruminococcus flavefaciens*

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University of Illinois, Department of Animal Sciences; Urbana-Champaign, IL 61801
Grant 99-35206-7950; $250,000; 3 Years

Metabolism and utilization of nitrogen by ruminant tissues during growth and production is completely dependent on nitrogen metabolism in the rumen. Ammonia is the major end-product of digestion of dietary protein, amino acids and urea as well as the major source of nitrogen for microbial protein synthesis in the rumen and indeed all gastrointestinal ecosystems. The proposed studies focus on the three primary enzymes of ammonia assimilation by rumen bacteria: glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate synthase (GOGAT). Consideration of the biochemical, genetic and regulatory processes involved in ammonia assimilation is critical and essential to achieve a balanced supply of protein and energy at the duodenum for nutrition of the ruminant animal. Importantly, more efficient capture of ammonia by bacteria in the gastrointestinal tract will reduce nitrogen loss in feces and urine and decrease environmental pollution.

The objectives of this proposal are to study metabolic and environmental regulation of GDH, GS, and GOGAT enzyme activity and corresponding gene expression in *Ruminococcus flavefaciens*, an important Gram positive, fiber degrading, bacterial species from the rumen and other gut ecosystems. Novel features concerning the activity, synthesis and expression of these enzymes will be studied using nuclease protection assays and *in situ* PCR to quantify mRNA using cells grown in continuous culture under varying ammonia concentrations and fluxes across the cell wall. Identification of differentially expressed mRNA will be determined to provide insight into the cellular mechanisms involved under conditions of nitrogen limitation or excess.

9903273 Muscle Cell Growth and Development

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Grant 99-35206-7951; $270,000; 3 Years

The proposed experiments will study muscle-forming cells and the regulation of muscle development and growth. Efficient muscle formation is central to successful agricultural production of meat. A protein known as Sca-1 is found only in cells that appear to be a special class of muscle-forming cells termed muscle stem cells. Muscle stem cells are progenitors of the cells which form muscle fibers. We will purify this small group of muscle cells and determine if they are indeed muscle stem cells. Also, we will examine how a group of proteins that regulate cell death affect muscle growth and development. One of these proteins, termed Bcl-2, appears to inhibit cell death and enhance muscle growth. We will examine cellular and molecular mechanisms by which Bcl-2 expression enhances the formation of skeletal muscle. One long-term goal of our work is to identify and purify muscle stem cells. There is currently no method to purify muscle stem cells. Our experiments could greatly increase our understanding of muscle growth. A second goal is to determine how Bcl-2 expression affects the quantitative outcome of myogenesis. These experiments in basic muscle biology will improve our understanding of muscle growth and development and could identify possible methods to improve muscle growth in agriculturally important animals.

9903272 FASEB Summer Research Conference on "The Calpain System in Health and Disease"

Goll, D.E.
Federation of American Societies for Experimental Biology; Bethesda, MD 20814
Grant 99-35206-7638; $15,000; 1 Year

This application is for support for a FASEB Summer Research Conference on "The Calpain System in Health and Disease". The objectives of the conference are: 1)To foster the development of intellectual and collaborative interactions among those individuals working on the calpains, who have come from a wide variety of backgrounds. 2)The conference will provide a forum for the discussion and development of new ideas and approaches to resolving some of the long-standing controversies in the calpain field. The calpain system is critically important to skeletal muscle growth and development, although the exact roles that the calpain system has in skeletal muscle are still unclear. It has been shown that calpain

activity is essential for fusion of embryonic skeletal muscle cells. Embryonic muscle lacks calpain activity resulting in no fusion of muscle cells to form mature muscle fibers and hence, no muscle tissue. Other studies have indicated that calpain activity is also essential for cell division or proliferation (impaired proliferation of embryonic muscle cells would lead to fewer cells to fuse and hence very small and/or very few muscle fibers in mature muscle). In growing animals, a variety of evidence has shown that the calpain system is involved in initiating turnover of the myofibrillar or contractile proteins. In this instance, inhibition of calpain activity leads to an increase in the rate of muscle growth. Clearly, new ideas on how calpain activity is regulated, both in developing muscle and in mature muscle fibers, has important implications to skeletal muscle growth and development. This conference will be limited to 140 individuals to facilitate intense and focused discussion of the problems and uncertainties facing the calpain system. It will be the first major international conference devoted to a discussion of the calpain system, and should promote development of new ideas in the calpain field.

9903365 FASEB Summer Conference: Ubiquitin and Intracellular Protein Degradation
Pickart, C.M.; Vierstra, R.D.
Federation of American Societies for Experimental Biology; Bethesda, MD 20814
Grant 99-35206-7671; $4,760; 1 Year

The conference “Ubiquitin and Intracellular Protein Degradation” will be held from July 31 through August 5, 1999 in Saxton’s River, VT. This meeting is the only regularly-held international meeting devoted to the biology and biochemistry of ubiquitin, a conserved protein ubiquitin which is essential for life in eukaryotes. The meeting is sponsored and partially funded by the Federation of the American Societies for Experimental Biology. The functions of ubiquitin are mediated by its attachment to other proteins in the cell. Its best-understood function is that of a signal which targets proteins for degradation by a novel protease. By setting the levels of key regulatory proteins, the ubiquitin pathway controls fundamental intracellular processes such as the progression of the cell cycle. The pathway also contributes to muscle wasting, a role that is likely to be relevant in large domestic animals. Besides ubiquitin, there exists a distinct family of ubiquitin-like proteins. These proteins seem to function as signals which target their substrates to specific locations within the cell. One notable member of this family, Nedd8, regulates cell cycle progression in mammals and auxin signaling in plants. A better understanding of the role of Nedd8 in the auxin response could allow for improved control of the growth of agriculturally important species. These and other topics will be addressed in the nine scientific sessions of the 1999 Conference. The Conference should substantially advance our understanding of the uniquitin system and its functions.

9903256 Role of Satellite Cell Activation and Growth Factors in Anabolic Steroid-Induced Muscle Growth of Steers
Dayton, W.R.; White, M.E.; Hathaway, M.R.
University of Minnesota; Department of Animal Science; St. Paul, MN 55108
Grant 99-35206-7935; $182,000; 3 Years

Anabolic-steroid-treatment of yearling steers enhances muscle growth. Our data suggest that both increased production of growth factors and a higher number of proliferating muscle satellite cells are involved in this anabolic-steroid-induced muscle growth. Circulating concentrations of the potent anabolic growth factor, IGF-1, and liver IGF-1 mRNA levels are significantly higher in anabolic-steroid-implanted steers than in nonimplanted steers. IGF-1 mRNA levels in the semimembranosus and longissimus muscles of implanted steers are higher than in the corresponding muscles of control steers. Thus, muscle tissue may produce higher levels of IGF-1 in anabolic-steroid-treated steers. A higher number of proliferating satellite cells can be isolated from the semimembranosus muscles of implanted steers than from the corresponding muscles of control steers. The ability of implantation to elevate circulating and muscle IGF-1 levels and to increase the number of proliferating satellite cells may play a critical role in anabolic-steroid-induced muscle growth in beef cattle. We propose to define the relationship between muscle levels of specific growth factor mRNAs, activation state of muscle satellite cells, and anabolic-steroid-induced muscle growth in beef cattle. These studies will increase our understanding of the mechanism of anabolic-steroid-induced muscle growth in an economically important species and will provide information about the potential role of satellite cell activation and specific growth factors in muscle growth.

9903371 Molecular and Kinetic Analyses of the Adherence of *Ruminococcus albus* to Cellulose
Morrison, M.
University of Nebraska, Lincoln; Department of Animal Science; Lincoln, NE 68583-0908
Grant 99-35206-8043; $250,000; 3 Years

Cattle and other ruminant animals depend on microorganisms to break down plant materials into a form which they can digest and use as nutrients. Cellulose is the most abundant polysaccharide consumed by forage-fed livestock, but for many enterprises, it is also the most poorly utilized part of the animals’ diet. Increasing cellulose degradation therefore offers great potential to improve nutrient retention and animal productivity. *Ruminococcus albus* is one of the three rumen bacteria coordinating the degradation of cellulose. Only a limited amount of information concerning the adhesion of these bacteria to cellulose is available, even though bacterial adhesion is believed to be a major rate-limiting step in cellulose breakdown. We

have discovered a new type of cellulose-binding protein produced by *Ruminococcus albus* (called CbpC), and also shown that the bacterium possesses a second mechanism for adhesion to cellulose. This grant focuses on characterizing the genetics and molecular biology underpinning these adhesion mechanisms. We will use a variety of recombinant DNA and biochemical techniques to learn which regions in these proteins are important for cellulose-adhesion, and how their capacity for adhesion might be altered. We also expect these studies will help determine microbial- and plant-borne constraints to improve cellulose degradation by *Ruminococcus albus*. Once these constraints are known, the potential to overcome them, and improve nutrient utilization in cattle will be maximized.

9903421 Molecular Mechanisms of Osmoregulation in Salmon

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Grant 99-35206-7936; $250,000; 3 Years

Osmotic stress is a constraint to commercial aquaculture of several species of fish. In salmon, one of the primary finfish cultured commercially in the US, this problem is particularly acute. In routine aquaculture practice, juveniles are transferred directly from freshwater hatcheries to netpens in full salinity seawater which can result in death and reduced growth rates of survivors. During our previous NRI grant period we found that heat shock proteins (hsps), a family of highly conserved proteins found in organisms from bacteria to humans, are expressed in Atlantic salmon exposed to seawater. Most striking, we demonstrated that subjecting juvenile salmon to a mild heat shock, capable of inducing hsps, enhanced short-term survival during subsequent exposure to hypersaline seawater by as much as 90%. We propose to extend this research to investigate the molecular mechanisms by which salmon adapt to seawater. First, the role of four specific genes in adaptation of salmon to seawater will be characterized. Second, the role of two naturally occurring compounds, betaine and taurine, in adaptation to seawater will be investigated. Preliminary studies in our laboratory suggest that uptake of these compounds into the cell is increased in salmon subjected to osmotic stress. Third, we will determine whether short term protection against salinity stress following mild thermal shock translates into increased long-term survival and growth of salmon following transfer to seawater. The research proposed here will investigate the basic mechanisms utilized by salmon to adapt to seawater and test the practical application of these results toward increasing production and economic gain to the aquaculturist.

9903266 Improving Flounder Productivity through Enhancing Growth

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Strengthening Award; Grant 99-35206-8585; $119,000; 3 Years

The United States imports $7 billion/year in excess of its seafood exports. In Europe and Asia, land-based flatfish farming has been a tremendous success and the immediate market for summer flounder in the United States is estimated at $20-30 million/annually. This year, for the first time, land-raised summer flounder were brought to market in the United States. A problem faced by growers is the variability in early growth rates. Because female flatfish out grow males by 2- to 4-fold, we propose to produce all-female crops of summer flounder. This is unusual because the effort in catfish and trout farming has been to produce male crops, which would support greater productivity. The likelihood of our success using estradiol treatment during a critical stage of gonad differentiation is high, based on parallel studies in model fish species. Rapid application of the assistance we propose would improve productivity and the cost:benefit ratio greatly, especially at this early stage in the industry's development.

9903265 Characterization of a Peptide Transporter Cloned from Chickens

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Grant 99-35206-7995; $135,483; 2 Years

Diets fed to poultry usually provide an excess of protein and an inadequate balance of amino acids. This is partly due to safety factors included by nutritionists and to an incomplete understanding of the digestion and absorption of these important nutrients. The result is that animal performance is less than optimal, feed costs are higher than needed, and the environment is contaminated by the excess nitrogen excretion. It is very likely that diets of poultry formulated to contain specific peptides, peptide mixtures, or blends of proteins will improve the balance of adsorbed amino acids. Intestinal absorption of amino acids as small peptides is recognized as being important in some animals. To understand how the nutritional management of dietary peptides can contribute to improved diets in poultry, more information is needed regarding the molecular mechanism of peptide absorption. The objectives of this project are 1)to determine the optimal composition and length of peptides transported by our cloned chicken peptide transporter and 2)to determine the influence that levels of dietary protein have on how much of the peptide transporter is present in different intestinal segments of broiler

chickens. This project will contribute to our understanding of the magnitude of peptide absorption and the nutritional significance of this process in poultry. This knowledge will lead to the development of recommendations that will enable poultry producers to optimize productivity.

9903253 Regulation of Amino Acid and Peptide Transporters in Ruminant Intestine
Knapp, J.R.
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Strengthening Award; Grant 99-35206-8044; $182,000; 2 Years

Nutrient utilization by the whole animal is a function of nutrient transport and metabolism by the epithelial cells lining the gut epithelium. The transport systems and metabolic activity of these cells have a major impact on whole body energy expenditures, protein turnover, and nutrient availability for other body tissues. Not only is an understanding of the basic processes at the epithelial level critical for efficient energy and protein utilization, but it is also key to protecting the environment. One of the largest issues facing animal agriculture is management of manure nutrients. Protein nutrition and amino acid utilization are critical to both growing and lactating cattle. The levels and activities of amino acid and peptide transporters in the small intestine of ruminant animals may be different from those of non-ruminants because of different flow rates of digesta into the small intestine, lower digesta pH for prolonged transit times, and the inclusion of microbial protein in ruminant digesta. The hypothesis to be tested in this proposal is that the structures and regulation of amino acid and peptide transporters in the small intestine of ruminants are similar to monogastric animals. A better understanding of how dietary and microbial proteins are digested and absorbed in ruminant livestock will lead to improved ration formulation, optimization of nutrient use for animal production, and reduced manure nutrient load released into the environment.

9903270 A Role for FGF6 in Regulating Satellite Cell Dynamics During Muscle Growth
Yablonka-Reuveni, Z.
University of Washington; Department of Biological Structure; Seattle, WA 98195-7420
Grant 99-35206-7934; $182,000; 3 Years

During embryonic development, muscle precursor cells fuse to form muscle fibers. The myofibers continue to enlarge following birth until the mature state is reached. A key element involved in postnatal muscle growth are the satellite cells which are situated by the myofibers. These cells can proliferate and produce differentiated progeny which fuse with the fibers. My research showed that several members of the fibroblast growth factor family (FGFs) can promote proliferation of satellite cells. However, only one FGF (named FGF6) also supports the differentiated state of the cells and is uniquely expressed by the myofibers. Hence, FGF6 might be a physiological regulator of muscle growth. The proposed studies will further examine the role of FGF6 during the proliferation and differentiation of the satellite cells. Isolated fibers from normal rats and mice, and from mutant mice lacking FGF6 will be studied. In this myofiber model, the satellite cells are retained in their native position associated with the muscle fiber. Results should, therefore, closely resemble the situation in living animals. The direct influences of FGF6 on satellite cells and on genes expressed by the entire myofiber unit will be monitored. Small laboratory rodents are used for the study because isolation of intact myofibers is only possible from these animals. Rats and mice have also provided an inexpensive experimental model for muscle growth which is applicable to agriculturally important animals. Understanding the mechanisms regulating muscle growth is crucial for improving the efficiency of muscle production by meat yielding animals.

9903363 Gene Therapy for Bovine Fatty Liver
Attie, A.D.; Grummer, R.R.
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Grant 99-35206-8045; $240,000; 3 Years

Fatty liver is a disorder that involves the accumulation of excess fat in the liver. The liver is normally involved in secretion of fat through particles called lipoproteins. In addition, in certain metabolic states, the liver receives a large amount of fat from other tissues, primarily from adipose tissue. If the influx of fat into the liver exceeds its ability to secrete the fat on lipoproteins, then fatty liver develops. Dairy cows frequently develop fatty liver shortly after they give birth. Fatty liver is associated with low milk production and can exacerbate many other disorders. Ruminants are especially vulnerable to fatty liver. This project is to explore the possibility that fatty liver can be ameliorated with gene therapy. Gene therapy involves the delivery of DNA to a tissue in an intact animal in such a manner as to allow the DNA to be used as a template for production of RNA and protein. We will test the ability of particular genes to alter the rate of lipoprotein production and thereby eliminate the ruminant's susceptibility to fatty liver.

ANIMAL GENOME AND GENETIC MECHANISMS

Panel Manager - Dr. Joan Burnside, University of Delaware, Newark
Program Director - Dr. Peter R. Brayton

The objective of this program is to increase our knowledge and understanding of the structure, organization, function, regulation and expression of genes in agriculturally important animals including aquaculture species. This includes but is not limited to: comparative gene mapping and the identification, isolation, characterization of genes, gene products and their regulatory mechanisms, identification and mapping of DNA segregation markers including quantitative trait loci (QTL), interactions between nuclear and organellar genes and the molecular basis of genetic replication, and development and application of methods to modify the animal genome. A new animal genome basic reagents and tools section was introduced this year to initiate: large scale EST sequencing, arrayed BAC libraries, DNA microarrays and high resolution genetic maps.

9903295 Arrayed Bacterial Artificial Chromosome Libraries of Agricultural Animal Genomes

de Jong, P.J.
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Grant 99-35205-8537; $570,000; 3 Years

During the last decade, the field of "Genomics" has come into existence following the declaration of the Human Genome Initiative by the U.S. Department of Energy and the National Institutes of Health. The main goal of this project is to analyze and understand the genomes of important species at the DNA sequence level. Our laboratory has highly focused on the development and implementation of large-insert genomic DNA cloning approaches. Through this specialization, we created Bacterial Artificial Chromosome (BAC) cloning vectors and generated large-insert BAC clone libraries for many species: vertebrates, invertebrates and single-cell organisms. BAC libraries are an essential resource to obtain a complete genome, and are very useful for transgenics, physical mapping, and comparative genomics. Precise matching of similar regions of the human genome and mammals of agricultural importance will accelerate comparative genomics. Through comparative genomics, agriculture will indirectly benefit from the extensive physical mapping and sequence information being obtained for the human and mouse. The goals of this project are to generate BAC libraries of the chicken, horse, cow, and pig genomes, characterize these libraries, and distribute them to agricultural animal research laboratories throughout the world. The availability of these libraries will help to create a genomic infrastructure for these species with BACs, for physical mapping, comparative mapping, and as a source of polymorphic probes for genetic mapping and for genome manipulation.

9903332 Mapping of the Pacific Oyster Genome

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Grant 99-35205-8260; $220,000; 3 Years

Farming of the Pacific oyster, *Crassostrea gigas*, on the West Coast accounts for more than one-third of U.S. oyster production and is important to rural economies suffering contractions in the fishing and logging sectors. The goal of this project is to discover genes controlling economically important traits in oysters, mainly growth and survival. The first step is to build a moderately dense linkage map, which permits genetic mapping of economically important traits. Over the past decade, we developed inbred lines for experimental study of the causes of hybrid vigor and of crossbreeding as a means of improving growth of farmed oysters. However, deviations from Mendelian rules of inheritance, commonly reported for bivalve molluscs but previously unexplained, impeded early efforts to map genes causing hybrid vigor. We recently showed that inheritance of markers is normal in 6-hr-old larvae but becomes distorted within two months by selection against recessive lethal genes linked to the markers. Lethal genes may cause much of the typically high larval mortality in commercial hatcheries. We are now poised to construct a genetic linkage map using nine reference families comprising tens of thousands of 6-hr-old F2 larvae, their parents and grandparents. We will develop ~150 anchor loci from non-expressed microsatellite DNA sequences. Trisomic oysters with one extra chromosome will enable assignment of lineage groups to specific chromosomes. Genes affecting growth will be mapped in large F2 populations, which have enormous variation in size. Finally, we will conduct inheritance studies to reveal the nature of lethal genes already detected.

9903339 Telomere Array Organization and Telomerase Function in Chicken

Delany, M.E.
University of California, Davis; Department of Animal Science; Davis, CA 95616-86671
Grant 99-35205-8369; $161,208; 2 Years

The enormous contribution of poultry to the competitiveness and profitability of U.S. agriculture dictates the need for a more complete understanding of the genomes of poultry species. The chicken is especially important because of the size of the U.S. commercial broiler and layer industries and because it serves as the paradigm for other commercially important poultry species. Knowledge of the structure, function, and stability of the chicken genome is essential for (1) continued progress in traditional commercial breeding by selection of superior genotypes and (2) purposes of genome manipulation, an area that is likely to be an important vehicle for future advances. Telomeres are the specialized ends of linear chromosomes maintained by telomerase. The dynamics of telomere length and telomerase activity directly impact genome stability by playing a critical role in cellular proliferation, senescence, and oncogenic transformation. The objectives of the proposed research are to: 1) map the location of particular telomere sequences within the uniquely-arranged chicken genome; 2) characterize the RNA and protein components of chicken telomerase; 3) investigate telomerase expression and function in maintaining telomere length in tissues derived from embryos and adults. The research will provide fundamental information regarding a critical genomic region that impacts differentiation, growth, aging and oncogenesis. The results will improve our understanding of the structure, organization, and function of the chicken genome and will contribute useful information for the selection of genomes for commercial purposes and biotechnology applications.

9903321 DNA Arrays to Monitor Host Gene Responses to Herpesvirus Infection
Schmidt, C.J.
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Strengthening Award; Grant 99-35205-8228; $199,961; 3 Years

The objective of this grant is to better understand the avian herpesvirus, Marek's disease virus (MDV). This virus causes both suppression of disease resistance and cancer in infected chickens. MDV is found in all large-scale chicken production facilities. Consequently, all commercially produced birds are immunized to prevent an outbreak of Marek's disease. While the disease is currently controlled by immunization, strains of MDV are emerging which are resistant to current vaccines. Thus, a better understanding of how to control Marek's disease is required. Our approach is to apply high-throughput gene screening to determine how the host responds to infection with MDV. By the use of DNA arrays containing thousands of genes, host gene expression that is either increased or decreased by MDV infection will be identified. The identity of the responding genes will be determined by rapid, automated DNA sequencing. The results may indicate how MDV suppresses disease resistance and induces tumors simultaneously. It may also provide insights to better control all herpesvirus infections.

9903296 Development of Resources for Functional Genomics in the Pig
Tuggle, C.K.; Rothschild, M.F.; Soares, M.B.; Casavant, T.C.; Prather, R.S.; Pomp, D.; Beavis, W.D.
Iowa State University; Department of Animal Science; Ames, IA 50010
Grant 99-35205-8370; $700,000; 2 Years

Advances in gene mapping and genomics of farm animals have been considerable over the past five years. First-generation linkage and physical maps in the pig have been instrumental in identifying chromosomal regions and candidate genes associated with traits of economic importance. However, current maps are not informative enough at the comparative level either for practical breed improvement or for studying biological mechanisms underlying economically important traits. An improved understanding of porcine reproductive biology is of crucial economic importance, yet reproductive processes are poorly characterized at the molecular level. Further, reproduction is very difficult to improve by classical breeding methods. The proposed project will address the basic need to identify thousands of genes associated with physiology of reproduction and to create the necessary infrastructure to share that information with other scientists. The Specific Aims of this research are to sequence a large number (20,000) of genes from porcine reproductive tissues, develop databases for public access, and map a significant number of these genes (700) which have been mapped in other species. All resources produced from this process (cloned DNA for each gene)will be publicly available and all data (sequence and mapping) will be accessible through an improved bioinformatics pipeline designed to allow interested scientists to make use of the information discovered. The proposed research will not only contribute to structural and functional genomics research that will improve our understanding of basic animal biology, it will also be directly relevant to the needs of the pig industry.

9903293 Production of 17,500 ESTs and Development of a High Resolution Radiation Hybrid Map
Lewin, H.A.; Womack, J.E.
University of Illinois; Urbana-Champaign; Department of Animal Sciences; Urbana, IL 61801-3838
Grant 99-35205-8534; $700,000; 3 Years

To address the present deficiencies in resources for cattle genomics, we propose to generate 17,500 3' expressed sequence tags (ESTs) from cattle cDNA libraries and to map 700 new ESTs on a bovine radiation hybrid (RH) panel. Results of this project will provide essential tools for identification of genes controlling traits of economic importance to the U. S. dairy and beef industries. We anticipate that this project will result in identification and mapping of approximately 7%

of all cattle genes. These genes will be a rich source of transcripts involved in reproduction, development, growth, metabolism and disease resistance. Mapped ESTs will be used for building detailed comparative maps, understanding genome evolution and leading the way towards molecular identification of genes affecting economically important traits. The maps, mapping tools and databases that will result from this research will significantly enhance the national effort in cattle genomics.

9903328 Coordinated Control of Pituitary Development and Function by Homeodomain Proteins
Rhodes, S.J.
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Grant 99-35205-8248; $190,000; 2 Years

The mammalian pituitary gland releases hormones that control growth, milk production, reproduction, body temperature, and the response to stress. The goal of this project is to study genes that regulate the function of the pig pituitary gland. The objectives include the identification and cloning of genes that are required for the development of the pituitary cells that secrete the hormones. In addition, the protein products of these genes will be studied and their biochemical properties characterized. Finally, the exact location of the genes in the DNA genome of the pig will be determined. This will allow investigation of whether the identified genes are associated with favorable agricultural traits, such as increased growth or reproductive capacity. By increasing our understanding of the genetic pathways that regulate growth, reproduction and physiology, these studies will guide future genetic, breeding and treatment protocols aimed at improving desired traits and productivity in agricultural species.

9903330 Production of Transgenic Fish by Nuclear Transfer from Cultured Cells
Collodi, P.; Barnes, D.W.
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Grant 99-35205-8186; $200,000; 2 Years

As seafood consumption in the United States continues to increase, a greater reliance is being placed on aquaculture to satisfy the demand for fisheries' products. To maximize yield and improve the efficiency of aquaculture production, a basic understanding of physiological factors controlling fish embryonic development, growth and survival is required. Also, genetic methods are needed that will enable us to efficiently alter characteristics of the fish such as growth rate, disease resistance and temperature tolerance in order to improve our ability to successfully grow fish in captivity. To address this problem we are working to develop a method that will allow us to introduce foreign genes into the fish genome resulting in the production of a transgenic fish that exhibits new genetic traits. Also, this method will allow us to destroy or knockout individual existing genes in the fish genome in order to produce a fish that lacks a single factor such as a particular hormone. The results from this work will enable researchers to study the role of specific physiological factors that are involved in regulating fish growth and survival and it will make it possible to alter genetic traits in order to make the fish more suitable for aquaculture production.

9903319 Extending the Life Span of Bovine Fibroblasts for Nuclear Transplantation
Jerry, D.J.; Robl, J.M.
University of Massachusetts; Department of Veterinary and Animal Sciences; Amherst, MA 01003
Grant 99-35205-8531; $250,000; 2 Years

Recent successes in cloning livestock from somatic (non-reproductive) cells has opened the possibility of producing animals with specific genetic characteristics. Although precise genetic alterations can be introduced into cultured cells by homologous recombination, cells derived from normal individuals eventually cease dividing before the selection process can be completed. Therefore, we propose to test methods to extend the cultured life span of primary cells. If successful, these methods will make it possible to produce animals with targeted placement of genes and to transfer desirable alleles from one genetic background to another.

9903351 Bovine IL-12 Receptor Gene Expression
White, A.M.
University of Massachusetts; Department of Veterinary and Animal Sciences; Amherst, MA 01003
Postdoctoral Fellowship; Grant 99-35205-8528; $89,547; 2 Years

Brucellosis, a disease caused by the intracellular pathogen, *Brucella abortus*, affects both humans and cattle. In cattle, it results in late spontaneous abortions and prolonged infertility. This is particularly important when considering dairy production, as aborting cows have decreased milk production. In addition, the loss of calves and the interruption of any breeding program is costly. Humans can contract brucellosis from ingesting unpasteurized milk or from handling an infected cow or aborted fetus. The disease manifests itself in several forms from artritis, nervousness, and uncontrolled shaking to undulant fever and meningitis. In an effort to better understand the immune response to this pathogen and aid in

the genetic improvement of cattle, I plan to study the bovine Th1 immune response by analyzing the expression of the interleukin 12 receptor known as IL-12Rb2. The Th1 immune response is particularly important in combating intracellular pathogens such as Brucella, Mycobacteria, and Listeria and is used to control protozoan parasites such as Cryptosporidia and Toxoplasma in some mammals. It has been shown in other mammals that immune response variations among individuals is controlled both by the ability of macrophages to make IL-12 and by the expression of the high affinity IL-12 receptor. I hypothesize that this will be similar with cattle. If true, then this knowledge will help create better vaccine cocktails to protect both animals and humans from these intracellular pathogens.

9903313 Identification of Single-Nucleotide Polymorphisms in Pig Sequence-Tagged Sites

Ernst, C.W.; Venta, P.J.
Michigan State University; Department of Animal Science; East Lansing, MI 48824-1225
Grant 99-35205-8150; $200,000; 2 Years

Our goal is to improve the pig and human comparative genome map by developing new sequence-tagged sites (STS) and by identifying single-nucleotide polymorphisms (SNPs) in pig STS. Comparative mapping allows the transfer of information from the human genome map to other species where resources for mapping are much more limited. Current genetic linkage maps for the pig contain very few Type I (i.e., gene) markers. The mapping of additional Type I markers on the genetic map and also on the recently developed pig radiation hybrid (RH) map will help to clarify gene order and significantly improve the human-pig comparative map. Our efforts will not only increase the density of the pig genetic and RH maps, but we will also improve the methodology for developing STS and identifying SNPs. Our specific aims include evaluating pig STS, identifying SNPs in pig STS and mapping pig STS using international mapping resources. STS will be physically mapped using a somatic cell hybrid panel and a radiation hybrid panel and informative SNPs will be used to locate STS on the European PiGMaP genetic linkage map. As results from quantitative trait locus (QTL) mapping projects are reported, the availability of high-resolution comparative maps will become increasingly important for identifying candidate genes responsible for genetic variation in economically important traits (i.e., the genes at QTL). Such information will benefit pork producers and U.S. consumers by enhancing our ability to efficiently produce high quality meat products.

9903300 An Integrated BAC Map of the Chicken Genome

Dodgson, J.B.; Zhang, H.
Michigan State University; Department of Microbiology; East Lansing, MI 48824-1101
Grant 99-35205-8566; $610,000; 3 Years

The proposed research aims to generate a nearly complete map of the chicken genome. The chicken genome is made up of 40 different DNA segments (chromosomes) which, in total, contain about 1.2 billion base pairs, the four letter code that determines all inherited traits. Recombinant DNA technology allows for the manipulation in bacteria of chicken DNA segments of 100-200,000 base pairs each, or about 200-400 segments per average chromosome. Our goal is to generate approximately 60,000 such overlapping segments and determine their exact order in the original chicken genome (for each of the 40 chromosomes). Once this work is completed, when a poultry geneticist locates any agriculturally-relevant inherited trait (by standard genetic mapping) s/he quickly will be able to determine a few overlapping cloned segments from our collection which must contain the gene encoding the trait of interest. (Eventually, each base pair (or letter) in the chicken's inherited code will be determined.) In this way, the physiological basis for inherited production traits will be better understood. That understanding can then be applied to improved management, nutrition and breeding strategies for egg and meat productivity.

9903307 Verification of QTL for Resistance to Marek's Disease in Commercial Chickens

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Grant 99-35205-8259; $220,000; 2 Years

Chicken is an important food source and, at 41% and growing, the primary meat consumed in the U.S. Primarily due to the utilization of advanced methods of selection in breeding programs, tremendous progress in production traits have been achieved to meet the growing demands of consumers in an economical manner. Recent advances in biotechnology may greatly complement traditional poultry breeding. Using molecular markers in genetic tests, elite progeny can be identified earlier and more accurately. With this goal in mind, we have identified in experimental chickens a number of genes that promote resistance to Marek's disease, the leading chronic disease of chickens and a $1 billion per year problem. The goal of this project is to transfer this information to commercial lines. In other words, we wish to determine if our genetic tests accurately predict disease resistance in elite commercial flocks. Success of this project will bridge the gap from experimental studies to commercial application, as well as enhance competitiveness of United States poultry breeding companies.

9903305 Bovine Gamma/Delta T Cell Expressed Sequence Tags

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Grant 99-35205-8618; $310,000; 3 Years

T cells expressing the gamma/delta (g/d) T cell receptor have been found in most vertebrate species, and they predominate in the blood of young ruminants, suggesting that they are particularly important for the immune defense of these animals. In contrast to alpha/beta (a/b) T cells, which primarily localize in lymphoid organs, g/d T cells are prominent within epithelial tissues such as intestine, lung and skin. This anatomical location at the portals of entry into the host has lead to the hypothesis that these cells may serve as an important first line of defense to infection. Though considerable effort has been devoted to the study of g/d T cells, only now is their importance to host immune responses being recognized, at least in rodents and humans. To further our understanding of the role of g/d T cells in cattle, we propose a large-scale EST approach to rapidly characterize the genes expressed by bovine g/d T cells. This will provide a catalog of the biochemical activities of bovine g/d T cells and provide the means for identifying novel or previously uncharacterized genes expressed in these important immune cells. In addition to facilitating our understanding g/d T cell biology, these ESTs will be placed on the bovine genomic map. The placement of coding loci (type I markers) is critical for extending the bovine database for evolutionary analysis of genome organization and for the identification of potential candidate genes associated with quantitative trait loci, in particular, those associated with disease resistance.

9903297 Generation and Mapping of Swine Microsatellites

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Grant 99-35205-8619; $420,000; 3 Years

The current porcine genetic map is sufficient for identifying regions of the genome, *i.e.* chromosomal positions, containing genes contributing to traits of interest or quantitative trait loci (QTL) in diverse populations. However, when regions of interest are identified generally there are insufficient genetic markers available to narrow the region of interest. Moreover, when commercial populations are examined, the number of gene variants (heterozygosity) decline when compared to the exotic breeds used to create the genetic maps. The levels of heterozygosity decline from 85%, to approximately 50%. Thus there is a need for more genetic markers for studies in commercial populations to identify QTL and narrow these chromosomal regions. This project involves the isolation of additional porcine genomic fragments containing polymorphic sequences termed microsatellite and the generation of synthetic DNA pieces that can be used via the polymerase chain reaction (PCR) to identify differences in swine under study. These new markers will be physically mapped using a relatively new technique called radiation hybrid mapping. This technique will permit us to rapidly place, resolve and order these new markers more efficiently than the traditional genetic mapping techniques. This information will be made available to the public by publication in scientific journals and the World Wide Web. The synthetic DNA primers will be made available to researchers worldwide to investigate porcine QTL.

9903292 Conference for the International Society of Animal Genetics

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Grant 99-35205-8557; $10,000; 1Year

Our primary objective is to organize the 27th International Conference on Animal Genetics, which will contribute to the discovery and dissemination of knowledge of animal genomics to serve food, agricultural, and ecological sectors of global societies. The International Society for Animal Genetics (ISAG) evolved from a series of annual workshops, to a society focusing on basic and applied research within the fields of immunogenetics, biochemical genetics, genomics and molecular genetics. The society's purposes are: (a) to encourage the study of genetically influenced characters of animal tissues and fluids; and (b) to facilitate the exchange of ideas and materials between research workers. In particular, the Society is proud of its international membership, which contributes diverse approaches to promoting animal genetics. Since 1954, ISAG has held its International Conference on Animal Genetics every 2 years at different locations around the world. Recent meetings in Tours, France (1996), and Prague, Czech Republic (1994), attracted over 500 scientists and students. Attendance at the year 2000 meeting is expected to be significantly greater than that of the very rewarding 1998 Auckland, New Zealand meeting (600), because of the meeting's accessible North America location. The aim of the meeting is to foster the interaction and exchange of information between all researchers in the field of genetics of farmed and companion animal species (from fish to horses); this is supportive of the aims of the recently developed U.S.D.A. Agricultural Genome Initiative.

9903341 Germ Line Modification in Farm Animals through Xenogeneic Spermatogonial Transplantation
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New Investigator Award; Grant 99-35205-8620; $300,000; 3 Years

Genetic modification of farm animals is of rapidly increasing importance in agriculture and medicine. The long-term goal of the proposed project is to develop a novel approach to generate transgenic farm animals through modification of the male germ line. In this project, we will recover male germ line stem cells from boars and bulls for transplantation into the testis of recipient mice where they are expected to initiate spermatogenesis of large animal donor germ cells. Experiments are designed to enhance donor cell proliferation and differentiation, and to enrich donor cell populations in stem cells before transplantation. Different culture conditions will be explored to optimize maintenance and proliferation of bovine and porcine germ line stem cells in culture as a basis for their genetic modification. Successful donor spermatogenesis after transplantation of frozen-thawed, cultured, or genetically altered germ cells will allow us to better understand the processes of spermatogenesis in farm animals and subsequently to improve fertility, preserve the genetic heritage of valuable donor animals, and make desirable genetic modifications in the male germ line. Transgenic farm animals expressing desirable genes will show improved production efficiency and disease resistance, reducing the need to use antibiotics and growth promotants which in turn will reduce the risk for residues in the human food supply. In addition, generation of genetically modified pigs as a much needed source of tissues and organs for transplantation to humans with advanced organ failure, and production of pharmaceutical substances in milk can provide potential means of alternate farm income.

9903336 Advanced QTL Analyses for Complex Traits in Pedigreed Populations with Application to Livestock
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Grant 99-35205-8568; $200,000; 3 Years

This project contributes to the goal of identifying some of the genes affecting traits of economic importance in livestock. Such traits (*e.g.*, milk yield and composition of dairy cows or carcass traits in swine) are typically quantitative and complex, *i.e.*, affected by multiple genes and environment. Identification of genes affecting quantitative, complex traits leads to a better understanding of the genetic basis and to more efficient manipulation of these traits needed for continued improvement of the productivity of livestock. Gene identification is based on two steps, linkage mapping which traces genes in current families, and linkage disequilibrium mapping which uses the history of a gene from the time the gene mutation occurred to the present. Here we develop appropriate statistical methods for both steps. Finally, marker assisted selection to estimate an individual's genetic value combines information from trait values (cows' milk production records) with information from genetic marker data. Genetic marker data are likely to be available only on current individuals (not on their ancestors) and only on elite animals (*e.g.*, nucleus animals). Consequently, a genetic evaluation system, which predicts the genetic value of each individual available for selection, must combine individuals with and without genetic marker data, and such a system is being developed here.

9903323 Cellular and Genomic Reprogramming of Different Cell Types by Nuclear Transfer
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Grant 99-35205-7998; $286,000; 3 Years

Cloning of cattle by nuclear transfer offers promise for genetic improvement of cattle. When donor cells are genetically engineered the promise of producing valuable proteins in their milk and acquisition of new genetic traits in the genome of cattle become possible. At present, the procedure is of low efficiency. We propose to improve the efficiency by understanding the process of genome reprogramming, a process necessary for offspring production. Our objective is to characterize the capability of different types of bovine donor nuclei (cumulus cells, fetal fibroblasts, adult skin fibroblasts and adult chondrocytes) to be reprogrammed by bovine metaphase II (MII) cytoplasm. This objective will be accomplished in four specific aims by experiments at the cellular (aim 1-2) and genomic (aims 3-4) levels as follows: 1) as indicators of cellular reprogramming, we propose to determine c-*kit* receptor and nuclear lamin expressions of nuclear transfer (NT) embryos derived from each cell type as well as; 2) transcriptional activities such as the ability of the embryo to turn on the embryonic genome and synthesize embryonic RNA; to determine the extent of reprogramming of the genome we propose to determine in aim 3) the pattern and timing of stage-specific gene expression as well as; 4) the DNA methylation pattern of bovine NT- produced embryos. These studies will be done in parallel with normal *in vitro* fertilized bovine embryos as controls on which the above determinations will also be made. We expect that these experiments will improve the outcome and success of cloning in domestic animals.

9903316 Estimation of Genetic Parameters Using Robust Linear Models

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Grant 99-35205-8162; $200,000; 3 Years

The objective is to develop statistical methods for analysis of genetic data in livestock that are less prone to aberrant observations and to errors in the assumptions made. These are called robust models. Procedures will be applied to lactation records of Holstein cattle from herds with complete recording on usage of bovine somatotropin (BST). At present, statistical methods for genetic evaluation of Holsteins do not include BST information. Because this hormone increases production, ignoring its effects can bias estimates of breeding values. Techniques for robust analysis of disease data will also be developed using mastitis records. This is the most important mammary gland disease in dairy cattle. Third, robust methods will be designed for application in gene mapping and use of molecular markers for genetic improvement of livestock. Fourth, a range of techniques will be developed for screening and comparing statistical models for quantitative genetic analysis of animal breeding information. It is hoped that the efficiency of animal agriculture can be enhanced through: 1) better statistical methods for identification of genetically superior animals; 2) improved techniques for genetic analysis of imperfect or biased data; and 3) developing foundations for more accurate identification and mapping of genes affecting variation for economically important traits.

ANIMAL HEALTH AND WELL-BEING

Panel Managers - Dr. Bradley W. Fenwick, Kansas State University; Dr. Mo Salmon, Colorado State University; Dr. Prem S. Paul, Iowa State University

Program Directors - Dr. Peter J. Johnson, Dr. Peter R. Brayton

The objectives of this program are to increase the knowledge needed to sustain animal health and well-being and to prevent or reduce the severity of animal disease. This includes, but is not limited to: mechanisms that alter the normal physiologic state at the molecular, cellular or organ level to produce disease resulting from either biotic or abiotic causes; cellular mechanisms of disease resistance, including developmental and molecular immunology; microbial genetics/ genomics; pathogenesis; both host and microbial factors influencing colonization of mucosal surfaces; host-environment or host-agent interactions that compromise host defense systems or cause predisposition to disease; epidemiologic studies on animal diseases that provide insight into etiologic factors and/or disease control; research that supports the development or evaluation of diagnostic tests and immunizations for emerging or reemerging disease problems such as tuberculosis; studies on economic models that address the costs of animal disease and the cost/benefit ratios of animal disease prevention and therapy. The program also encourages research on the mechanisms controlling animal responses to physical and biological stresses (including quantitative behavioral, physiological, immunological and neurobiological responses to stress) and the development of objective indicators to measure animal well-being.

9902123 Mitochondrial Dysfunction in Pulmonary Hypertension Syndrome in Broilers

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Grant 99-35204-8617; $200,000; 3 Years

Pulmonary hypertension syndrome (PHS) is a metabolic disease in poultry that costs U.S. companies over $100 million annually. Due to inherent problems in oxygenating blood, a series of physiological and metabolic events occur in fast growing chickens (broilers) that cause death from congestive heart failure. We have evidence that cellular oxygen use, specifically within mitochondria (where most oxygen consumption occurs), is inefficient in broilers with PHS. This inefficient oxygen use may be due to leakage of electrons (from the electron transport chain of mitochondria where cellular respiration occurs) that react with oxygen to form reactive compounds that can oxidize critical cellular components (e.g. lipids and DNA). Therefore, a major goal of the proposed studies will be to determine the causes of mitochondrial dysfunction in PHS. In these studies, lung and liver mitochondria will be isolated from broilers with and without PHS as well as from broilers that are genetically susceptible and resistant to PHS. Mitochondrial dysfunction will be assessed by measuring oxygen consumption, peroxide generation (an indirect measure of electron leak), and proton leak in isolated mitochondria. Electron leak will be assessed with chemical inhibitors that block electron transport at specific sites of the electron transport chain. If defects in electron transport, electron leak, and antioxidant protection and/or mitochondrial DNA function can be identified, these will serve as guides for genetic selection methods to remove PHS-susceptible birds from the commercial population. If this can be accomplished, producers and consumers may benefit from reductions in the overall costs of producing poultry meat.

9902303 Mode of Action of Pyolysin in *Arcanobacterium pyogenes* Virulence and Host Protection

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Grant 99-35204-7818; $262,500; 3 Years

Arcanobacterium pyogenes is a common opportunistic pathogen of domestic animals. In the cattle industry, it is a significant cause of mastitis and contributes to the formation of liver abscesses. We have previously demonstrated that the potent, *A. pyogenes* toxin, pyolysin (PLO), is an important virulence factor in *A. pyogenes* infections, due, in part, to its ability to kill host immune cells. Therefore, we hypothesize that the role of PLO in *A. pyogenes* pathogenesis is to interfere with the host immune function, allowing an infection to become established. This hypothesis will be examined by assessing the ability of PLO to interfere with various immune parameters, including host cytokine expression, inhibition of phagocyte chemotaxis, respiratory burst, and complement activation. In addition, the ability of wild type and PLO mutant *A. pyogenes* to survive or evade phagocytosis will be studied. PLO is also an efficacious vaccine in protecting mice for *A. pyogenes* infection. However, because of its toxicity, we have constructed genetic toxoids of PLO with reduced cytolytic activity which will be assessed for their ability to protect mice from experimental *A. pyogenes* infection. These studies will then be extended to the field, where a PLO based vaccine will be tested in feedlot cattle in an attempt to reduce the formation of severe liver abscesses. Liver abscessation is a significant economic disease in intensively grain fed beef cattle, resulting in decreased feed efficiency and carcass dressing percentage.

9902013 Bluetongue Virus Infection of Cattle: Maximal Duration of Infectious Viremia

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Grant 99-35204-7863; $190,000; 3 Years

Bluetongue remains a costly impediment to US agricultural trade (particularly of cattle and germplasm) and a continuing threat to the health of domestic and wild ruminants throughout much of the country. Precise definition of the maximal duration that bluetongue virus (BTV) infectious to insect vectors (*Culicoides variipennis sonorensis*) circulates in the blood of cattle following infection clearly is prerequisite to further rationalization of trade policies pertaining to bluetongue. The molecular evolution of BTV in endemic regions of the US also is poorly characterized, especially the influence of selective pressures exerted on individual viral proteins during sequential passage of BTV through its insect and ruminant hosts. We propose to: 1) determine the duration of infectious viremia in cattle infected with BTV by the bite of BTV-infected *C.v. sonorensis* insects, using both standard diagnostic procedures and insect feeding studies; 2) directly sequence key genes of BTV during sequential passage of the virus through the ruminant and insect hosts. Genes encoding BTV proteins responsible for virus attachment to mammalian (VP2) and insect (VP7) cells will be evaluated, as will those (NS3/3A) responsible for cellular viral egress. The overall goal is to use information derived from these studies as the basis for the development of more rational trade policies pertaining to international trade of ruminants and their germplasm, and to initiate studies to identify the selective pressures that cause periodic emergence of novel strains of BTV in the U.S.

9902298 Role of Type III Secretion in *Bordetella bronchiseptica* Pathogenesis

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Grant 99-35204-7680; $300,000; 3 Years

Bordetella bronchiseptica is a bacterial respiratory pathogen that infects a broad range of mammals. Together with *Pasteurella multocida*, *B. bronchiseptica* causes infectious atrophic rhinitis in swine, a serious and widespread disease responsible for significant economic loss to the pork industry. As a primary cause of tracheobronchitis (kennel cough) in dogs and snuffles and bronchopneumonia in rabbits, *B. bronchiseptica* also imparts an economic impact on animal breeding, boarding, and training industries as well as laboratory animal medicine facilities. *B. bronchiseptica* infections are notoriously chronic in nature and difficult to eliminate with antibiotics. Moreover, despite the availability of more than 10 vaccines on the market, none has been shown to be particularly efficacious, thus, *B. bronchiseptica*-mediated diseases persist.

B. bronchiseptica expresses several virulence factors, including a "Type III Secretion system" which was discovered in our laboratory. Type III secretion systems function like molecular syringes, allowing Gram-negative bacteria like *B. bronchiseptica* to inject proteins into host cells. Once inside, the bacterial proteins can interact with host cell proteins resulting in altering function or even death of the host cell. Our preliminary experiments suggest the Bordetella type III secretion system may contribute to the chronic nature of *B. bronchiseptica*-mediated diseases by altering the function of cells involved in the development of a potent immune response. We propose to identify, characterize, and evaluate the functions of factors that are secreted by the Bordetella type III system. Results obtained will significantly increase our understanding of pathogenic mechanisms and facilitate the development of live attenuated, hyperimmunogenic vaccine strains effective against *B. bronchiseptica* and possibly against *P. multocida*.

9902087 Mitigation of Disease in Feedlot Cattle Caused by 3-Methylindole, a Rumen Generated Toxin

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Grant 99-35204-8616; $205,000; 3 Years

Bovine respiratory disease (BRD) is an important cause of reduced performance of feedlot cattle. While BRD has been recognized as a classical example of multi-factorial disease, toxins have not been considered important in the disease process. Sudden, dramatic increases in rumen generation of 3-methylindole (3MI), a normal product of tryptophan metabolism, have been recognized as being important in the pathogenesis of acute bovine pulmonary emphysema and edema (ABPE). However, 3MI has not been considered important in the development of BRD of feedlot cattle. We hypothesize that oxidative damage caused by 3MI enhances development of BRD. There is evidence that cattle experience more severe discase when they are concurrently exposed to respiratory pathogens and 3MI, that increased 3MI production is associated with decreased rates of gain in feedlot cattle, and that feeding aspirin and vitamin E may be useful and economical for prophylaxis of 3MI-associated disease. The specific aims of the proposed research are to: 1) demonstrate that low level oral aspirin administration can protect cattle from lung damage in part through blocking conversion of 3MI to toxic metabolites; and 2) evaluate the potential benefits of feeding aspirin and vitamin E during the peak periods of 3MI generation on rates of gain, feed intake, feed efficiency, carcass characteristics, disease occurrence, and lung damage in feedlot cattle. Our long-

term goal is to provide cattle feeders with an inexpensive regimen that will improve productivity, decrease the impact of BRD, and help decrease reliance on antimicrobial drugs in agriculture.

9902077 Sequence Analysis of the Herpesvirus Associated with Malignant Catarral Fever
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Grant 99-35204-7723; $187,000; 2 Years

Malignant catarrhal fever (MCF) is a highly fatal disease of cattle, bison, deer, and other bovids. It is characterized by high fever and disease involving several organ systems. Historically MCF has been known primarily as a sporadic disease, although isolated epizootics have been described in zoos and in feedlots and, more recently, in commercial bison herds in the Western U.S. The etiologic agent of the sheep-associated form of the disease appears to be a herpesvirus identical with or closely related to the Rhadinovirus ovine herpes virus 2 (OHV-2). Since this virus has not been cultured *in vitro*, it must be studied as a DNA sequence, but less than 1% of its nucleotide sequence has been determined to date. 1 kb has been sequenced so far. This project will focus on the Rhadinovirus found in MCF-affected cattle and bison in the Western U.S. We propose to sequence potentially variable regions of the genome that contain genes probably responsible for viral pathogenesis. The results of this research will be pivotal to future studies that will clearly define the etiology of MCF in cattle, bison, and deer, define the epizootiology of MCF virus(es), provide a molecular basis for the pathogenicity of OHV-2, and point the way toward novel vaccine-based and other strategies to control this important disease.

9902023 Functional Analysis of Marek's Disease Virus Latency
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Grant 99-35204-7948; $300,000; 3 Years

Marek's disease virus (MDV) is an oncogenic herpesvirus that latently infects T-lymphocytes and induces T-cell lymphomas following infection of susceptible chickens. Marek's disease is regarded as one of the most serious infectious diseases of poultry. It is controlled by vaccination of commercial chickens worldwide and its continued control is vital to the sustainability of the poultry industry. Fundamental studies on Marek's disease are needed to improve vaccines and to develop novel disease control strategies. The goal of this research is to understand the molecular details of latency and its relationship to lymphoma formation. We are particularly interested in the interactions between viral and cellular gene products that no doubt occur in infected cells. Previously we have reported the discovery and characterization of RNAs expressed during latency (latency associated transcripts) that are anti-sense to the ICP4 gene, a gene whose expression is critical for MDV productive infection. We have also isolated a mutant virus that fails to normally express these RNAs. Experiments are planned to characterize this mutant with regard to latency and lymphoma formation and to assess global cellular and viral gene expression changes that accompany latency.

9902326 Extracellular Protease: Potential Virulence Factor of Mycoplasma Mycoides
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Grant 99-35204-8367; $160,000; 2 Years

Mycoplasmas, a type of bacteria, are of major world-wide economic significance in diseases of cattle and goats. Although it is true that small ruminants are a minor component of the U.S. agricultural livestock base relative to cattle and poultry, they nevertheless represent a significant economic contribution. In 1996 goat meat import/export resulted in a trade deficit of $5 million. Clearly the demand in the U.S. exceeds the current production, and the small ruminant industry, if adequately supported, could become an important component of U.S. agriculture. Most mycoplasmal infections cause clinically silent, chronic disease, but the infections associated with young goat kids have an acute and often fatal clinical picture. The death rate in affected kids can be high (sometimes > 90%), and the economic losses devastating. We hypothesize that a major contributing factor to this disease is the production of a protease (an enzyme which breaks down protein) by the mycoplasma. This protease could play a role in the increased spread of mycoplasma to the joints and brain of the goats as well as in the excessive edema or fluid associated with pneumonia and joint damage seen in arthritic manifestations. The specific objectives of this proposal are: 1) to develop a series of mycoplasmal strains which do not produce the protease; and 2) to see if goats infected with these strains have less severe disease. If our hypothesis is correct, then new treatments which could ultimately decrease deaths and losses due to mycoplasmal disease may be pursued.

9902336 Molecular Mechanisms of Enteric Lipopolysaccharides (LPS) in Horses: Antagonism by Rhizobial LPS
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Grant 99-35204-7790; $190,000; 3 Years

Endotoxemia is associated with the leading causes of death in horses and foals due to gram negative bacteria. Thus, a clear understanding of the molecular events involved in endotoxemia together with the identification and characterization of agents that can interfere with these events would significantly impact animal health. In other species it has been shown that the pro-inflammatory effects of endotoxin are mediated though two proteins, a specific plasma protein (LPS-binding protein) and a cell surface receptor protein (CD14) that function synergistically to enhance the ill effects of endotoxin. However, it has not been possible to study the role of these mediators in the horse as equine-specific reagents that detect LPS-binding protein and CD14 have not been available. Thus, our objectives include developing these reagents by purifying equine serum LPS-binding protein and expressing recombinant equine CD14. With these essential tools in hand, our second objective will be to determine the relative efficacy of certain, structurally-novel, rhizobial lipopolysaccharides to antagonize the deleterious, proinflammatory effects of endotoxin on equine mononuclear cells. Therefore, these studies have potential to lead to the development of a new therapeutic agent while simultaneously shedding light on the molecular mechanisms involved in equine endotoxemia.

9902454 Prevention and Treatment of Fatty Liver in Dairy Cows by Glucagon

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Grant 99-35204-8576; $195,000; 3 Years

Dairy cows are susceptible to metabolic and infectious diseases in early lactation. One significant metabolic disease is fatty liver, a major accumulation of fat in the liver, which usually occurs in the first 1-3 weeks after calving. Some estimates suggest that up to 50% of U.S. dairy cows have mild to pathological fatty liver in early lactation, costing millions of dollars in treatment and production losses. Fatty liver seems to cause increased incidences of other disorders such as ketosis, metritis, mastitis, and decreased reproductive efficiency. Currently, pathological fatty liver can neither be prevented consistently nor treated effectively by nutrition or any other means. However, we recently published evidence that glucagon, an important mammalian hormone, can be used to greatly speed up removal of fat from livers of affected dairy cows. Our long-term goal is to provide veterinarians and dairy farmers with glucagon as an effective tool to prevent or treat fatty liver and to thereby improve the health and well-being of U.S. dairy cows. Our objective is to determine which of two dosages of glucagon is most effective at two and eight days after calving to remove or decrease the accumulation of fat in the liver and to identify metabolic processes involved in the beneficial effects of glucagon. Prevention and treatment of fatty liver will avoid millions of dollars in financial losses for dairy farmers. The results will lead to management options that enhance the health and life expectancy of dairy cows and improve the sustainability and profitability of dairying.

9902079 Antigen Specific T Lymphocyte Responses to Type 1 and Type 2 Bovine Viral Diarrhea Viruses

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Grant 99-35204-7899; $295,000; 3 Years

Bovine Viral Diarrhea Virus (BVDV) infection is the most significant viral cause of economic loss to the cattle industry in the United States. The virus infects cattle of all ages and causes economic loss by inducing enteric, respiratory and reproductive disease. The currently available killed-virus vaccines have limited ability to provide cross protective immunity against a broad range of BVDV isolates and give a relatively short duration of immunity. The modified live vaccines protect against a broader range of BVDV isolates and induce longer term immunity. However, they are not safe for use in stressed animals, pregnant animals, or calves and heifers exposed to pregnant cows. This project will allow the investigators to characterize the differences in the immune response induced by killed and modified live vaccines against the two major types of BVDV. The investigators will clone individual genes from Type 1 and Type 2 BVDV isolates into a vaccinia virus carrier, and use these new viruses to determine which ones induce the most effective type of immune response and the most effective cross protective immunity. The proposed research has the potential to generate basic information that could lead to the production of new genetically engineered vaccines that could provide optimal safety and efficacy. Such a vaccine could have a significant impact in reducing the loss associated with BVDV infection in cattle.

9902314 Virulence Mechanisms of Bacterial Pathogens International Symposium

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Grant 99-35204-7720; $9,000; 1 Year

An international symposium entitled Virulence Mechanisms of Bacterial Pathogens will be held September 12-15, 1999 in Ames, Iowa. This meeting will provide an overview of current knowledge of the wide variety of mechanisms used by bacterial pathogens in establishing infection, producing disease, and persisting in the host. Strategies for overcoming bacterial virulence mechanisms will also be presented. The emphasis will be on understanding the mechanisms of host pathogen interactions rather than on current research results. A monograph based on this meeting will be published by the

American Society for Microbiology. The monograph will be entitled Virulence Mechanisms of Bacterial Pathogens, Third Edition, 2000. All meeting registrants will receive a copy of the monograph.

The symposium will be organized under the following topics: Bacterial invasion, colonization, and survival; Bacterial evasion of host defense mechanisms; Bacterial effects on host cell function; and Identification, regulation and transfer of virulence genes. Each major topic will be divided into overview presentations by internationally recognized authorities in the field. Attendees are invited to submit abstracts for poster presentations. The symposium will provide information for molecular biologists, infectious disease researchers, industry scientists, faculty and graduate students interested in bacterial pathogenesis.

9902323 Antimicrobial Peptides: Ontogeny and Production by Ovine Respiratory Epithelia

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Grant 99-35204-7681; $180,155; 2 Years

Bacterial pneumonia is common in ruminants. *Pasteurella haemolytica* is a frequent cause of pneumonia in sheep and is an important pathogen of cattle. The mechanisms by which *P. haemolytica* evades innate respiratory mucosa defense are poorly understood. Antimicrobial peptides (AMP) are a part of the innate host mucosal defense that are becoming increasingly appreciated. AMP, produced by the respiratory mucosa and leukocytes, have potent bactericidal activity and also antimicrobial activity against certain viruses, fungi and parasites. The ontogeny and mechanisms of AMP production are poorly understood, as are the cell types of the respiratory mucosa that produce AMP. It is our hypothesis that certain AMP (-defensin and cathelicidin) can be induced prior to or during *P. haemolytica* colonization of the ovine respiratory tract and, thereby reduce the severity of infection. In these studies we will establish a profile of AMP production in respiratory tract mucosa from nasal cavity, trachea, bronchi and lung from ovine fetuses, neonates and adults. We will determine whether or not enhanced differentiation of cultured ovine respiratory cells by a novel water-soluble form of vitamin A enhances production. This work will establish a baseline profile of the ontogeny of AMP production in the respiratory tract of sheep.

9902043 Role of Gut Colonization in the Response to TI, TD and Mucosal Immunogens

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Grant 99-35204-8469; $280,000; 3 Years

The first six weeks of life are a critical period in the survival of newborn farm animals. Agricultural productivity is directed correlated with the number and health of the offspring. Thus, determining what factors are important during this critical period is a priority in animal health research.

Very early in life the gastrointestinal (GI) tract of newborn mammals, birds and many other animals is colonized by a commensal, non-pathogenic bacterial flora. Since the GI tract together with the respiratory tract presents the largest surface area of the body for sampling the environment, colonization of the GI tract can be expected to influence the development of the immune system of the newborn. Such development is implied since newborn piglets reared in a germfree (GF) environment die when later exposed to a conventional environment while “conventionalized” newborn (those allowed to become colonized) do not.

The proposed research investigates the role of colonization on development of the neonatal immune system of the piglet using a variety of state-of-the-art methods that allow development of the immune system to be measured at the molecular level including at the level of single cells. Specifically, we wish to determine if colonization significantly improves the piglet’s ability to mount an immune response to the different types of antigens that are constituents of most neonatal vaccines. Since bacteria that colonize the GI tract are believed to do so using certain adhesion proteins, the capacity of bacteria with such proteins or variants which lack them, will be tested for their ability to influence the development of the neonates immune system and to enhance immune responses to the major types of antigens.

While the work will generate considerable basic information on developmental immunity, it should also provide a framework for the development and administration of neonatal vaccines.

9902346 Gordon Conference, Biology of Spirochetes

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Grant 99-35204-7787; $9,000; 1 Year

The fourth Gordon Research Conference on the Biology of Spirochetes will be held in January 2000 in Ventura, California. This conference is the only ongoing international meeting devoted to basic research of all medically important and biologically relevant spirochetes. This group of bacteria cause a number of diseases, notably digital dermatitis of cattle, leptospirosis, dysentery in humans and swine, syphilis, Lyme disease, relapsing fever, periodontal disease, and possibly

epizootic bovine abortion. As a group, these organisms have historically been difficult to study because many are notoriously difficult to grow in culture, and systems by which their genetics can be studied are in the very early stages of development or are nonexistent. This conference will bring together representatives from laboratories studying all aspects of spirochete biology in an informal forum to discuss cutting-edge research developments. Topics to be included for discussion include physiology, structure, pathogenesis, and immunobiology of these bacteria. The goals of the conference are to foster an environment where current research collaborations are strengthened and new collaborations are developed, and to help define new areas of focus for research in this field. Special attention will be payed to the adequate representation of young investigators at the graduate student, postdoctoral, and junior faculty levels, as well as an appropriate balance in terms of gender and ethnicity.

9902318 Immunosuppression by Host-Specific Staphylococcal Enterotoxin C in Mastitis
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Strengthening Award; Grant 99-35204-8581; $180,000; 2 Years

Staphylococcus aureus is a bacterium that causes important diseases in humans and animals. Bovine mastitis is one infectious disease caused by this bacterium with a major economic impact for producers. Despite many attempts to control staphylococci in dairy herds, mastitis remains a major problem. Our hypothesis is that toxins produced by staphylococci in the mammary gland suppress the bovine immune response and prevent elimination of the organism. We have shown that one common toxin, staphylococcal enterotoxin C (SEC), produced by many mastitis strains, causes the bovine immune system T cells to function in a way that could cause immunosuppression. The goals of this present study are two-fold. First, experiments will investigate the ways in which SEC causes a relative decrease in helper T cells. This work will identify cellular targets of the toxins and provide information that could lead to methods for protecting the animal. The second objective is to determine the types of toxins involved in bovine mastitis. This objective is important since staphylococci are known to produce at least 10 different types of toxins with properties similar to SEC and several new toxins have been discovered in recent years. We will survey at least 500 staphylococcal isolates from various geographical areas around the world for toxin production. Combined, these studies will define the role for certain types of bacterial toxins in bovine mastitis. If this study implicates immunosuppressive toxins in mastitis, new targets for treatment and prevention will be made available to the dairy industry.

9902295 *Pasteurella multocida*-Related Toxins: Structure & Activity
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Grant number pending; $280,000; 3 Years

Atrophic rhinitis, caused by the toxin-producing bacteria *Pasteurella multocida* and *Bordetella bronchiseptica*, is a serious, economically important disease in the swine industry. The purified protein toxins from these bacteria alone can reproduce many of the clinical manifestations of the disease in animals, such as snout deformities, decreased weight gain, and small body stature. Certain bacterial strains of *Escherichia coli* that cause enteric disease in cattle produce a related protein toxin. These diseases adversely influence the market value of the animals and result in significant economical losses to producers. Understanding the mechanism of how the toxins cause cytotoxicity to cells could potentially lead to novel therapeutic and preventive measures against the diseases caused by these veterinary pathogens. The proposed studies are aimed at carefully dissecting the functional parts of this group of related toxins and comparing their structural organization so as to gain insight into the mechanisms of their action. Previous studies conducted on the toxin from *Pasteurella* will be expanded upon and extended to include comparison with the other two toxins. Deletion mutants of the three toxins will be expressed under controlled conditions in mammalian cells to delineate the function of the conserved regions of the proteins. Mutants containing toxin fragments linked to a green fluorescent protein will be used to identify via fluorescence microscopy regions of the proteins responsible for binding and entry into cells.

9902018 Receptor Therapy for Porcine Group A Rotavirus Disease
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Grant 99-35204-7982; $298,000; 3 Years

Group A rotaviruses are ubiquitous species-specific pathogens. In all species of domestic mammals in which they have been described they produce diarrhea. Illness due to rotavirus infections in neonatal and post-weaning pigs and calves often reaches 80% while death losses can be as high as 60%. The national Pork Producers Council recognizes enteric disease of pigs as the most costly disease problem facing pork producers; rotavirus is the pathogen most often associated with diarrheal disease of both nursing and weaning pigs. Although recent vaccine studies have resulted in an apparently effective modified live vaccine for protection against human rotavirus disease, similar success with porcine rotavirus is unlikely. Live

attenuated rotavirus vaccines have previously been shown to lack efficacy in pigs in the field. Our approach has been to focus on the initial recognition event between the virus and host cell; a step required for establishing infection and disease. Through previous NRICGP funding we were the first to identify, isolate and characterize a porcine intestinal receptor for rotavirus. The receptor is a type of glycolipid called a ganglioside and is required for virus attachment to cells. We also demonstrated the purified receptor, when added back to host cells in soluble form, could protect the cells from virus attachment and infection. Subsequent receptor characterization studies allowed us to define the specific chemical components and spatial orientation properties of the ganglioside receptor required for inhibition of rotavirus attachment and infectivity. In a previous study it was also shown that depletion of gangliosides in whole animals using an inhibitor of their synthesis, blocked ganglioside receptor function without causing deleterious effects on the animal. Finally, we have developed a unique *in vitro* assay for measuring assembly of infective rotavirus particles and discovered a new sugar-binding activity in VP6, an inner core viral protein. The knowledge gained from these basic studies have now allowed us to begin to propose novel therapeutic strategies for control or elimination of porcine rotavirus disease. The development of these strategies is the subject of this proposal. We propose to: 1) optimize the use of natural enterocyte receptor molecules or their active derivatives as *in vivo* competitive, receptor mimetic inhibitors of rotavirus attachment to host cells; 2) determine the chemotherapeutic effect of *in vivo* depletion of enterocyte CM3, using inhibitors of ganglioside biosynthesis, on porcine rotavirus disease; and 3) determine the role of our newly discovered VP6 sugar-binding activity in virus assembly or maintenance of virus; 4) establish the molecular basis for virus outer capsid assembly and evaluate the preclinical feasibility of using soluble carbomimetic and/or *in vivo* depletion of enterocycte gangliosides as a therapeutic modality in the prevention/treatment of porcine rotavirus disease.

992051 The Role of Type 2 Cytokines in Equine Recurrent Airway Obstruction (RAO)

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Grant 99-35204-8516; $200,000; 2 Years

Chronic obstructive pulmonary disease (COPD) and summer pasture associated obstructive pulmonary disease (SPAOPD), together referred to as recurrent airway obstruction (RAO), are two of the most common respiratory diseases affecting horses worldwide. While upwards of 10% of horses in North America may be affected, little is known regarding the aeropathogenesis of these diseases. Recent work indicates that both diseases are immunologic in nature and may be similar to asthma in humans, but the predisposing factors leading to this condition are unknown. We propose that gastrointestinal parasites may predispose horses to developing these diseases by providing an environment in the horses' lung conducive to the development of these diseases. To test this hypotheses we propose the following objectives: (1) to further characterize cytokine production in respiratory system of RAO horses; and (2) to determine the effect of parasitism on the immune response to aerosol challenge with a novel antigen. The first phase of this project will be to collect clinical and immunological data on a group of SPAOPD, COPD and control horses to more fully characterize cytokine mRNA levels in their respiratory system. The second phase involves using a novel antigen to induce RAO in both parasite-free and parasitized ponies and to determine the possible role parasitism may play in the initial development of this disease. By better understanding the underlying immunological mechanism for these diseases, it may be possible to identify those horses at risk for these diseases and develop better therapeutic and prevention strategies.

9902131 Mucus Secretion and Clearability in Equine Chronic Obstructive Pulmonary Disease

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Grant 99-35204-8366; $220,000; 3 Years

Chronic inflammation of the air passages (chronic obstructive pulmonary disease, COPD) affects 33 percent of horses in training and cripples many older pleasure and performance horses. In this disease, the airways become obstructed by spasm of the muscles and by accumulation of mucus. It is not known if accumulation of mucus is a result of excessive production or of defective removal or both. We have developed the tools to examine both the quantitative (mucus secretion) and qualitative (mucus physical properties) aspects of airway mucus in horses. We will use these tools to determine: 1) if increases in mucus secretion are always associated with airway inflammation; 2) if excessive secretion or altered physical properties persist after inflammation has waned; 3) which products of inflammation are responsible for increased secretion; 4) if horses with chronic airway disease produce a different type of mucus that is more difficult to remove from the airways; and 5) the degree to which mucus is responsible for airway obstruction. The results of this collaborative study between Michigan State University and the University of Alberta will allow veterinarians to better advise the horse industry on management and treatment of airway disease. An additional benefit will be the development of diagnostic tools for identification of susceptible animals and methods for screening the efficacy of drugs used to treat equine chronic airway disease.

9902137 Bovine Mucosal Immune Responses to *Cryptosporidium* Infection

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Grant 99-35204-8614; $295,000; 3 Years

Cryptosporidiosis, caused by the intestinal protozoan *Cryptosporidium parvum*, is now recognized worldwide as an important disease of humans and animals of agricultural interest. At the present time, there is no effective therapy for treating or preventing cryptosporidiosis. Active cryptosporidiosis is self-limiting in immunocompetent hosts and results in partial or complete protection against subsequent infections. This lack of basic understanding of the molecular mechanisms involved in the host's immune response against *Cryptosporidium* has been a limiting factor in designing effective means of treating and preventing disease. The intent of the proposed studies is to provide a basic understanding of the nature of the host's leukocyte response to *Cryptosporidium* infection in cattle. These studies will take advantage of our ability to directly cannulate the lymphatic vessel draining the ileal tissues in calves. This novel experimental system will allow us to characterize the development of immunity during a primary infection, and the kinetics of the acquired immune responses to a challenge infection within a single animal. By identifying the specific host cells responsible for recognizing and clearing primary infections, and understanding the mechanisms involved in developing resistance to subsequent infection, future therapies for treating and preventing cryptosporidiosis can be designed based on the biology of the specific effector cells.

9902068 Infectious Recombination System for Porcine Reproductive and Respiratory Syndrome Virus

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New Investigator Award; Grant 99-35204-8185; $183,938; 2 Years

Porcine reproductive and respiratory syndrome virus (PRRSV) causes great economic losses to the pork industry through its ability to produce respiratory and severe reproductive disease in swine. However, little is known about the mechanisms which PRRSV uses to produce these effects. The overall goal of this study is to define a new genetic system to investigate these mechanisms. Former research has developed two ways to genetically manipulate viruses to determine genomic regions of virulence and to study viral pathogenesis, but problems have been encountered with both of these methods. In the proposed study, we will attempt to blend these two methods by preparing DNA clones of overlapping segments of the PRRSV RNA genome, producing RNA copies of the cloned DNA and introducing these RNA copies to host cells. We will then investigate the possibility that new whole virus, which is indistinguishable from natural PRRSV, will be derived by a process called viral recombination. If successful, this proposal will result in a novel and fundamental tool with which to analyze individual properties of the PRRSV genome and may open up avenues of investigation for many other viral pathogens of animals.

9902047 Non-Antimicrobial Action of Macrolides: Role in Modulation of the Inflammatory Response

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New Investigator Award, Grant 99-35204-8411; $87,000; 1 Year

Bovine respiratory disease (BRD) impacts all areas of the cattle industry with cost estimates ranging from 600 - 800 million dollars annually. Improved outcomes are reported by employing effective management, vaccination and antibiotic strategies. Disease remains a major concern, since stress and microbial pathogens play synergistic roles in disease. Treatment is aimed at reducing the growth of bacterial organisms, and controlling the host inflammatory response. Controlling inflammation is key to reducing losses associated with BRD. Several approved drugs modulate the inflammatory response within the lung in addition to killing bacteria.

The proposed research will examine the cellular mechanisms that antibiotic agents employ in the modulation lung inflammation. Specifically, macrolides modulate inflammation by cellular mechanisms involving cAMP and protein kinases. One macrolide, tilmicosin (Mictotill9), modulates inflammation via inducing inflammatory cell death, while minimizing damage to the lung. We will define these mechanisms in the absence of infection by determining the responses of cells isolated from the lung and peripheral blood of animals treated with macrolide antibiotics. We will characterize the responses of these cells to stimuli by defining intracellular signaling, cell mediator production, and functional changes in inflammatory cells. More complete understanding of the inflammatory response will provide needed information to allow development of therapies aimed at more precisely controlling inflammation, and reducing the use of drugs in food animals.

9902097 Attenuated *Salmonella* Antigen and DNA Vaccine Delivery Vaccines Against *Eimeria*
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Grant 99-35204-8572; $195,000; 2 Years

Our objective is to design and fully develop two new means to deliver DNA vaccine vectors and antigens to chickens to induce immunity to prevent coccidiosis, a disease caused by a parasite which causes bloody diarrhea with high mortality and poor performance by survivors. Coccidiosis is currently controlled by the addition of drugs to poultry feed, which is costly and also decreases performance of chickens in terms of rate of growth and conversion of food into meat. Our approach will make use of attenuated strains of *Salmonella typhimurium* as the vectors for delivery of the DNA vaccines or coccidiosis antigens to young chicks prior to the time that they have contact with parasite eggs. *S. typhimurium* is normally a pathogen. The strain to be used will have mutations due to deletion of segments of DNA so that the resulting strain, although able to infect chickens and induce immunity, is unable to cause any disease either in chickens or in other animals, including humans. The same attenuated strains of *S. typhimurium* have been used to: develop a live vaccine to prevent *Salmonella* transmission from chickens to humans; and to display antigens from strains of *E. coli* which cause a respiratory disease in chickens that results in high mortality and condemnation of carcasses at the processing plant. The current research will thus expand and extend our ability to use attenuated *S. typhimurium* strains to control important diseases in agronomically important animals, in this case poultry.

9902042 Gene Discovery in *Neospora caninum*
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Grant 99-35204-8615; $300,000; 3 Years

Infection by the parasite Neospora is a significant cause of abortion developmental defects in cattle. While not a direct threat to human health, these infections result in considerable economic losses. Development of effective vaccines or treatments to prevent neosporosis will require a more complete understanding of this parasite. One of the main limitations is our lack of knowledge about the genes, or sequences of DNA, that enable the parasite to infect cattle and cause disease. To address this problem, we will generate a gene sequence database for the parasite Neospora using semi-automated technology to analyze the expressed genes of this parasite. This snap-shot approach can be accomplished very rapidly and economically and the DNA sequences will be made available to all interested investigators. Comparison of these sequences with the existing gene databases will identify genes that may be important targets for prevention of infection. The rapid availability of this information should significantly boost research productivity on parasitic diseases in livestock by fostering further research on diagnostic markers and possible vaccine candidates.

9902967 Identifying Genes Encoding Protective Antigens for Enteric Septicemia of Catfish
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Grant 99-35204-7721; $96,000; 2 Years

Edwardsiella ictaluri is the bacterium that causes enteric septicemia of Catfish (ESC), the most devastating disease to channel catfish aquaculture. Control of ESC will require effective vaccines. Early vaccines for ESC were ineffective partially because they were made of killed bacteria from artificial culture systems. In the catfish, *E. ictaluri* grows within the white blood cells and the proteins that are produced in artificial systems may not include important ones produced during an infection. Also, the way that the immune system of the catfish identifies and reacts to the proteins from the killed bacteria is not the same as the way it does proteins produced from live bacteria during an infection. We have developed an effective vaccine vector using the channel catfish virus. We believe that expressing bacterial proteins in our vaccine vector system would provide an effective vaccine for ESC. To make this vaccine, we first need to identify the proteins of the bacterium that induce a protective immune response in the catfish. Our goal is to identify the genes in *E. ictaluri* that code for these proteins. To do this, we will first select genes encoding proteins that antibodies from ESC survivors can identify. The potential of the respective proteins to vaccinate catfish against ESC will be evaluated by injecting catfish with DNA containing these genes. Then, after three weeks, the catfish will be challenged with ESC. Fish injected with the genes encoding proteins that induce protective immunity will have better survival than those injected with other genes.

9902343 Role of *Edwardsiella ictaluri* Lipopolysaccharide in Disease Pathogenesis
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Strengthening Award; Grant 99-35204-7679; $125,000; 2 Years

Channel catfish farming has become an important agricultural industry, with over 5.6 million pounds of farm-raised catfish processed in the U.S. during 1998. The most serious disease affecting farm-raised channel catfish is enteric

septicemia of catfish (ESC), which is caused by *Edwardsiella ictaluri.* At the Fish Diagnostic Laboratory in Stoneville, Mississippi, which serves an area containing the largest number of catfish farms in the U.S., *E. ictaluri* was isolated from 41.2% of catfish cases submitted for disease diagnosis in 1998. The purpose of this study is to gain a better understanding of the outer polysaccharide coat of *E. ictaluri* and the role it plays in causing disease. The polysaccharide coat of *E. ictaluri is* composed of long chains of repeating sugar subunits, termed lipopolysaccharide. In other bacterial pathogens, lipopolysaccharide plays an essential role in disease by forming a protective coat around the bacteria, allowing them to resist certain components of the host immune response. Previously published research on *E. ictaluri* has already provided evidence that its polysaccharide coat is important in the disease process. In this study, we will clarify the role of *E. ictaluri* lipopolysaccharide in the disease process by determining how it affects specific essential components of the channel catfish immune response. We will also study how lipopolysaccharide affects the overall disease process in channel catfish. By gaining a better understanding of the role *E. ictaluri* lipopolysaccharide plays in disease, we may be able to develop an improved vaccine or a new treatment for ESC.

9902968 Confirmation of the Myxozoan Life Cycle Associated with Proliferative Gill Disease in Channel Catfish
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Grant 99-35204-8387; $135,000; 3 Years

Proliferative gill disease (PGD) is a disease that is responsible for major fish losses in catfish. PGD is associated with *Aurantiactinomyxon ictaluri*, a parasite which uses the aquatic oligochaete Dero digitata, as an intermediate host. In challenge studies, we infected catfish with *A. ictaluri*. PGD organisms were observed in the gills 7 days later. The remaining fish were sampled 3 months after infection and a myxozoan cyst stage was found. Spores in this cyst stage were purified, DNA was isolated and the rRNA gene was amplified using PCR primers. The gene sequence of the spores was identical to the gene sequence of *A. ictaluri*. This data provides supportive evidence that this cyst stage is the final fish stage in the PGD life cycle. If that is the case, then we should be able to infect *D. digitata* with the spores from the cyst and demonstrate an infection of *A. ictaluri* in *D. digitata*. We should, in turn, be able to isolate *A. ictaluri* from the worms, infect catfish and demonstrate PGD organisms and the cyst stage, thus completing the life cycle. Using our *A. ictaluri* specific primers, we propose to: confirm each life stage of PGD in laboratory experiments; do a controlled study to simulate the life cycle of PGD in the field and finally use PCR primers to identify the fish population that is the primary source of this parasite in the catfish industry. This information will be used to control or eliminate this parasite in catfish.

9902019 Identification and Molecular Characterization of Cytotoxic Cells in Catfish
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Grant 99-35204-7844; $263,000; 3 Years

Channel catfish are the most important aquacultural species in the United States and are a significant component of the economies of several states. However, as aquacultural practices become more intensive, the likelihood of serious disease among farm-raised catfish increases. The long-range goal of this proposal is to enhance disease prevention through an expanded understanding of the catfish immune system. Specifically, this project will focus on anti-viral cell-mediated immunity (CMI) because, although little is known about CMI in fish, in other animals CMI is essential for recovery from virus infections. The goals are threefold: (1) to demonstrate that catfish possess authentic cytotoxic T cells; (2) to characterize natural killer and other cytotoxic cells; and (3) to elucidate the genetic organization and function of catfish major histocompatibility complex (MHC) class I genes. To achieve the first aim, MHC matched 2nd generation gynogentic fish will be used to determine if catfish utilize MHC-restricted, cytotoxic T cells to kill virus-infected targets. Successful completion of this study will facilitate the assessment and development of vaccines designed to induce both antibodies and cytotoxic effector cells in catfish. Specific aim #2 will provide fundamental information on the mechanisms of cell-mediated killing by different types of channel catfish cytotoxic cells. Finally, since currently little is known about catfish MHC and its role in immunity, the third aim will provide important information on the genetic organization and role of catfish MHC genes and gene products in cell-mediated immunity. Collectively, these studies will provide the fist evidence that fish utilize cell-medicated immunity to control virus infections and provide the theoretical basis for vaccines designed to enhance this important component of immunity.

9902122 Gamma/Delta T Cell Chemokines
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Grant 99-35204-8269; $300,000; 3 Years

The trafficking of immune cells through out the body is required for effective host immune responses against pathogens. δT cells are the predominant immune cell in newborn calves and are likely important to the immunological

health of the animal. To date, little is known about the function of these cells. The intent of this project is to continue our investigation of the molecular basis for the unique trafficking patterns bovine δT cells follow. In the past, we have focused on adhesion events at the vessel surface, including migration across the endothelial barrier after adhesion has taken place. In this new line of investigation we intend to define soluble factors (chemokines) which direct δT cell migration out of vessels and into tissues, and begin to determine how these factors coordinately regulate the specificity of the migration process. The results from these studies will contribute significantly to our overall understanding of bovine δT cells and how they may be potentially regulated to the ultimate benefit of the animal. For example, new adjuvant formulations might be developed that use chemokines to selectively attract and stimulate these cells.

9901997 Mucosal Immunity to K99 Fimbriae Encoded by *Salmonella:* Vaccine for Bovine Enterotoxigenic *Escherichia coli*

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Grant 99-35204-7773; $175,253; 2 Years

Live vector delivery systems represent an effective means to vaccinate mucosal surfaces for the induction of mucosal and systemic immunity. In this effort, we propose to use attenuated *Salmonella typhimurium* strains that are adept in delivering vaccine antigens to lymphoid inductive sites, e.g. the Peyer's patches of the gastrointestinal (Gl) immune system. Developing vaccines against any number of diarrheal livestock diseases can amount to significant economic benefit for livestock producers. To further our understanding of rudiment immunity, we have developed modified *Salmonella-based* vaccines to express the K99 fimbrial protein from enterotoxigenic *Escherichia coli* (ETEC). This is one of the etiological agents responsible for scouring disease common in newborn calves. One of the problems in developing efficacious vaccines for cattle is the lack of understanding how to induce either antibody or cell-mediated immunity. To address this problem, we have developed three *Salmonella*-based vaccines to test how placement of the K99 fimbrial protein within or on the cell surface of the *Salmonella* can stimulate different host immune responses. In doing so, we will learn more about the bovine immune system and how variation in antigen presentation impacts bovine antibody and immune T cell responses. Ultimately, these studies could lead to better design of livestock vaccines for the development of protective immunity.

9902292 Pathogenesis of the Fin Ulceration Response (FUR) in Fish

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Grant 99-35204-7722; $200,000; 2 Years

Disease is a major obstacle to successful aquaculture. Fish often become sick and develop skin infections when they are stressed, such as when they are handled, shipped, or placed into confined spaces such as holding tanks. We have discovered that when sunshine bass are placed in a confined space, they will slough the skin on their fins, producing an ulcer. This ulcer forms within 2 hours or less after they are confined. This Fin Ulceration Response (FUR) explains why many microbes which are always in the water can suddenly cause a skin infection. We suspect that FUR may play an important role in causing many of the most common bacterial and fungal infections of fish.

In this project, we will determine if administering a combination of stress hormones (adrenaline, noradrenaline and cortisol) will cause FUR. We will examine the microscopic changes that occur with FUR. We will determine if fish that develop FUR are more susceptible to skin infections. We will also determine if FUR allows the invasion of microbes into the internal organs of the fish, which has relevance to fishes' resistance to many diseases, as well as the effectiveness of bath vaccines. Finally, we will determine if stress can cause FUR in channel catfish and rainbow trout. Our work should help to identify the types of infectious diseases which may develop as a consequence of FUR and provide a first step in eventually understanding how to control this possibly very widespread problem.

9902312 Construction and Analysis of Virulence Mutants in *Bordetella avium*

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Grant 99-35204-7743; $200,000; 3 Years

The objective of the research is to better understand the pathogenesis of avian *bordetellosis*, particularly in commercial turkeys. *Bordetellosis* is an upper-respiratory tract infection caused by the gram negative bacterium *Bordetella avium*. The disease is prevalent in all the major poultry production regions of the world. Studies have shown evidence of *B. avium* infection in 80-100% of surveyed non-commercial domestic poultry and wild turkeys. Infected turkeys become more susceptible to infection with other pathogens that can produce mortality. The economic impact to the turkey industry is significant. The genetic traits that make *B. avium* virulent are not well defined. We propose three ways to discover and better understand such traits: (i) We outline experiments to exploit a new technique that identifies avirulent *B. avium* mutants. From the study of these mutants, we hope to better understand the factors that make *B. avium* virulent. (ii)

Bacterial surface organelles are often important for attachment to airway epithelium. We have discovered the genes for two potentially important attachment organelles: *fhaB*, which encodes a filamentous hemagglutinin, and *fimB*, a gene necessary for fimbrial production. We investigate the role of these factors in pathogenesis. (iii) Investigate the role of lipopolysaccharide (LPS), a cell bacteriophage (bacterial viruses). *B. avium* mutants spontaneously resistant to the phage are often avirulent. By studying the properties of the phage-resistant mutants, we hope to better understand factors contributing to virulence.

9902116 Immunization Against Bovine Cryptosporidiosis with Protective Antigen

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Grant 99-35204-8533; $150,000; 3 Years

Cryptosporidiosis is an economically important diarrheal disease of newborn calves caused by *Cryptosporidium parvum*. Cryptosporidiosis is a public health concern as well, since *C. parvum* infects humans. The resulting disease is life-threatening in hosts with defective immune systems. Currently, there are no specific preventive or treatment modalities approved to combat the disease.

We have cloned a gene of *C. parvum* with demonstrated ability to induce protective colostrum following immunization of cows. The immune colostrum protected calves against cryptosporidiosis and reduced oocyst excretion by 99.8%. Our proposed three year study is designed to evaluate three independent immunization strategies employing DNA vaccines that stimulate immune responses against this efficacious recombinant antigen. We will test the hypothesis that single dose administration of recombinant *Cryptosporidium parvum* induces colostrum that protects against cryptosporidiosis. The hypothesis will be tested via the following objectives: 1) determine whether or not oral immunization with recombinant *C. parvum* cDNA induces colostrum that protects against cryptosporidiosis; 2) determine whether or not recombinant *C. parvum* C7, administered intramuscularly with IL-4 DNA or IL-12 DNA, induces colostrum that protects against cryptosporidiosis; 3) demonstrate that the best immune colostrum protects calves following oral challenge with *C. parvum* oocysts.

Upon successful completion of this targeted three year study, we will have validated an immunization strategy for protection of calves against disease caused by *C. parvum*. The end result will be satisfactory control of a disease that has emerged as an important livestock health and public health concern in the United States and the world.

9902316 Identification of *Mycobacterium paratuberculosis* Virulence Determinants

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Grant 99-35204-7789; $210,000; 2 Years

Paratuberculosis, or Johne's disease, is a granulomatous enteritis of ruminants which is prevalent in those areas of the United States with high concentrations of cattle. The dairy industry loses over $1.5 billion every year because of this disease. *Mycobacterium avium* subsp. *paratuberculosis* is the causative agent of Johne's disease. Key to its virulence is its ability to grow and survive in phagocytic cells. Important questions about how bovine macrophages interact with this bacterium remain unanswered. We have discovered that growth hormone and prolactin also influence the ability of *M. paratuberculosis* to replicate within macrophages. Using a bank of *M. paratuberculosis* transposon mutants, we propose to screen these mutants in bovine macrophages with novel methodologies aimed at identifying genes important for intracellular bacterial replication and pathogenesis. We expect these studies to lead to the identification of *M. paratuberculosis* virulence determinants, and ultimately the development of improved vaccines, diagnostic tests and control strategies.

9902027 Genetic Analysis of Elements Controlling Bovine Viral Diarrhea Virus Translation

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Grant 99-35204-5068; $180,000; 2 Years

Cattle and calf death losses in the U.S., largely consequence of infectious diseases, amounted to $1.8 billion in 1995. Losses due to reproductive disease are not included in this statistic but could exceed this figure. BVDV infections are responsible for major losses in all sectors of the cattle industry due to their role in reproductive losses, respiratory and enteric diseases, and high fatalities in calves. Such losses due to a viral infection that is preventable by vaccination seem illogical. Vaccines are not widely used because they are perceived as either unsafe or ineffective. The need for more effective tools to control BVDV; i.e. vaccines or therapeutics, has never been more pressing. The work proposed seeks to identify regulatory elements which control protein synthesis by the viral genome. These elements are essential to identify virulence determinants to be manipulated in the engineering of live attenuated BVDV vaccines. We are confident that the newly developed BVDV reverse genetics approaches will allow us to capitalize on the newly identified regulatory elements, by understanding the impact of viral determinants of virus pathogenicity. Ultimately, this information should provide targets

for a rational approach for BVDV control. Survival of pestiviruses in herds hinges upon their ability to cause fetal infections leading to persistent infection. Vaccines which prevent transplacental transmission f BVDV would allow implementation of control and eradication plans to preserve the profitability of cattle farms.

9902016 The Effect of Porcine Reproductive Respiratory Syndrome Virus on the Immune System During Acute and Persistent Infection

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Grant 99-35204-8041; $150,000; 2 Years

Porcine Reproductive Respiratory Syndrome Virus (PRRSV) causes the most economically significant infectious disease of domestic swine in the U.S. For the last three years our research has focused on identifying the type of cells affected by PRRSV in infected swine and also on how this virus persists inside the body of animals that survive an acute infection. Our research indicates that the persistent infection established by PRRSV is limited in time and that the load of infectious virus progressively declines until this virus disappears completely from the body of the affected animals.

We also found evidence for a selective delay, suppression or modulation of humoral (neutralizing antibodies) and cellular components of the PRRSV-specific immune response, which we postulate may be those important for prevention of the infection. We propose to continue these studies by pursuing the following two objectives:

1) Is the observed delay in humoral neutralizing immune response of any consequence for the overall protection (or lack of thereof) against PRRSV? To this end, we will confirm if neutralizing antibodies have a role in protection by conducting experiments of passive transfer of humoral immunity in pregnant sows.

2) Can we identify a direct viral effect or an indirect effect of PRRSV infection on immune cells that would explain this apparent modulation? We will study the individual cells of lymph nodes in PRRSV infected animals and look at possible direct replication of PRRSV in lymphocytes, frequency of PRRSV specific lymphocyte populations, and lethal damage or inflammatory alterations in single cells.

9902030 Epidemiology and Etiology of Salmon Swimbladder Sarcoma Virus

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Grant 99-35204-7845; $195,000; 3 Years

In 1997 approximately 35% of the Atlantic salmon collected from the Pleasant River, ME, and maintained in captivity as brood stock, developed swimbladder sarcomas, a clinical pathology never seen in North America. An identical disease, affecting pen-reared Atlantic salmon, occurred in Scotland in 1976. A retrovirus, tentatively named salmon swimbladder sarcoma virus (SSSV), has been molecularly cloned from tumors and part of its DNA sequence determined. The information available to date suggests that SSSV is most likely the cause of the swimbladder tumors as it is present at high levels in these diseased tissues. At the North Attleboro Hatchery, MA, where the disease outbreak occurred, 52% of the salmon were positive in a blood-cell-based PCR test. Disease-free stocks of Pleasant river salmon, collected independently and maintained at a commercial facility showed an infection level of 5%. We hypothesize that salmon swimbladder sarcoma is an emerging retroviral disease that is endemic to natural and captive salmon populations. To address this hypothesis, three aims are presented that investigate the genetic structure, prevalence, and mechanism(s) of spread. First, to determine the complete nucleotide sequence of SSSV which will allow for the development of sensitive diagnostic tools; second, to determine the prevalence of SSSV in natural and captive populations of salmon and other fish that co-habitate with salmon; and third, to assess the mechanism of natural transmission of SSSV and induction of swimbladder sarcoma.

9902025 Basis of Increased Virulence of Newly Emerged Asian Foot-and-Mouth Disease Virus

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Grant 99-35204-7949; $251,866; 2 Years

Foot-and-mouth disease (FMD) is economically the most important viral disease of farm animals, the number-one foreign animal disease threat to the US, and the most significant disease impacting free trade in animal products. Current USDA policy dictates that a US outbreak would be controlled by quarantine, slaughter, disinfection, and ring-vaccination. Recently FMD has been controlled in Europe and parts of South America through vaccination of cattle, reducing changes of introduction to the US through movement of products and people from these regions. However, in the Spring of 1997, a devastating outbreak of FMD occurred in Taiwan. Control of the resulting epizootic resulted in destruction of approximately 4 million swine, and had an estimated economic impact of 5-7 billion dollars. Interestingly, the virus causing the outbreak (O1 Tai) displays altered virulence relative to well-characterized European and South American viruses; high morbidity and mortality in swine, and inability to infect cattle. We have recently identified changes in the O1 Tai viral genome that are responsible, in part, for its restricted ability to infect bovine cells in culture. This project will define the genetic basis for

growth restriction of O1 Tai in bovine cell cultures, define the genetic basis for altered virulence in cattle and swine, and determine the origin of the altered genome of O1 Tai. The results of these studies will help to define how this frightening new virus strain emerged, and provide new information on the control of FMD if it were to be introduced into the United States.

9902134 Isolation and Characterization of *E. risticii* from Trematodes

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Grant 99-35204-8521; $175,000; 3 Years

Potomac horse fever (PHF) is a life-threatening disease of horses characterized by fever, loss of appetite, depression, and watery diarrhea. PHF is caused by a small bacterium called, *Ehrlichia risticii*, which infects blood leukocytes and cells lining the gut wall of the horse. How the horse acquires this bacterium is still unknown. The long-term goal of this research project is to identify where and how *E. risticii* exists in nature, and how the horse gets infected. Studies indicate that the disease is not contagious and vector-borne. Recent studies suggest that trematodes in the fresh-water snail harbor *E. risticii*. Ehrlichial genes were identified by using a very sensitive and specific laboratory method in a specific type of fluke infesting a specific type of snail collected from stream waters in central Ohio and northern California where PHF frequently occurs. We propose to: (1) isolate live *E. risticii* from these flukes by mouse inoculation and cell culture; (2) clone fluke isolates and sequence 3 different genes of *E. risticii* isolates and compare these sequences with those of local horse isolates; and (3) inoculate selected fluke isolates of *E. risticii* into horses to examine their infectivity and pathogenicity. These approaches will permit us to prove the existence of live *E. risticii* in flukes, which are infectious and potentially pathogenic to the horse. Therefore, the proposed research will provide much needed information to develop preventive strategies for limiting exposure, thus limiting use of ineffective vaccines and dependence on antibiotic therapy.

9902009 Porcine Enteric Calicivirus: Molecular Analysis and Synthetic RNA Transcripts

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Grant 99-35204-7900; $195,000; 3 Years

Enteric caliciviruses cause diarrhea in swine (porcine enteric calicivirus) and humans (human calicivirus), but there is little information on the prevalence of porcine enteric caliciviruses in swine or their genetic relationships to one another or to human caliciviruses. It is important to establish the relationships between human and swine enteric caliciviruses because human caliciviruses are now the leading viral cause of food-borne gastroenteritis. However, no diagnostic assays are available to detect porcine enteric caliciviruses or antibodies to porcine enteric caliciviruses in swine. We will develop sensitive RT-PCR assays to survey for porcine enteric calicivirus infections in swine and the strains detected will be sequenced to assess their genetic relatedness to one another and to human caliciviruses. In addition we will create synthetic infectious RNA transcripts for our cultivable porcine enteric calicivirus strain to study the molecular basis for *in vitro* replication and growth restrictions of porcine enteric caliciviruses. Such studies should identify the genetic regions responsible for cell culture adaptation of these fastidious enteric caliciviruses and may be applicable to cell culture adaptation of the fastidious human caliciviruses, none of which grow in cell culture.

9902337 Role of a Multidrug Eflux System in Antibiotic Resistance of *Pasteurella haemolytica*

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Grant 99-35204-7678; $100,000; 2 Years

Bovine respiratory disease (BRD) is an important cause of death and disease in cattle and represents a significant economic loss to the cattle industry. *Pasteurella haemolytica* is considered the primary bacterium involved in BRD. Antibiotics are used therapeutically and prophylactically to treat cattle with BRD and reduce the incidence of BRD, respectively. Their use accounts for a significant portion of the costs associated with control of BRD. The widespread use of antimicrobials for the prevention and control of BRD suggests that antibiotic resistant *P. haemolytica* can have significant economic impact. Recently, systems that function to export numerous antibiotics from bacteria have been identified. These multidrug resistance (MDR) systems are now recognized as serious problems in the treatment of infectious diseases. We have identified a new potential MDR system in *P. haemolytica*. The *P. haemolytica* MDR system, like similar systems in human pathogenic bacteria, may mediate clinically relevant resistance to antibiotics. The goal of this project is to determine if the *P. haemolytica* MDR system functions to mediate multiple antibiotic resistance like similar systems do in human bacterial pathogens. By comparing mutant bacteria strains with the normal wild-type strain, we will determine the contribution of the MDR system to multiple antibiotic resistance in *P. haemolytica*. We will examine a wide array of

antibiotics to determine which are less effective at killing *P. haemolytica* because of the MDR system. The results of our study can provide new insights into the emergence of antibiotic resistance among bacterial pathogens of animals.

9901988 Analysis of Host Proteins that Bind to the Translocated Intimin Receptor of Enterohemorrhagic *Escherichia coli* and Enteropathogenic Escherichia coli

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Grant 99-35204-7864; $260,000; 3 Years

A serious problem for animal health results from bacterial infection by two groups of diarrheagenic *Escherichia coli*: Enteropathogenic *E. coli* (EPEC) and Enterohemorrhagic *E. coli* (EHEC). These bacteria attach to the mucosal linings of farm animals, causing diarrhea and leading to the contamination of the meat and other food products produced by the animal. In order for the bacteria to attach to the animal's intestinal cells, a bacterial protein (Tir) must be inserted into the membrane of the infected cells. The attached bacteria disrupt the cytoskeletal architecture of the host cells and the normal functioning of the intestine. The aim of this proposal is to identify proteins inside the host cells that interact with Tir in order to determine how the actin cytoskeleton is disrupted by EPEC and EHEC. We will use the yeast two-hybrid system to screen a library of genes to identify proteins that can bind to Tir, followed by functional and biochemical assays to examine the binding properties of candidate proteins with Tir. We will also determine if a synthetic peptide that mimics the part of Tir that binds outside the host cell to EPEC/EHEC will block bacteria binding to host cells. Identification of interactions between animal proteins and the bacterial protein, Tir, will provide basic information needed to perturb those interactions and the accompanying pathology that occurs when these bacteria colonize mucosal surfaces.

9902327 Fimbriae-Mediated Adhesion of Enterotoxigenic *Escherichia coli*

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Grant 99-35204-8013; $300,000; 3 Years

Specificity of the initial adhesive contact between a pathogenic microorganism and its animal host determines whether an infection can proceed. Identification and characterization of the adhesive components on microbes and their receptor counterparts in hosts are instrumental for developing preventive and therapeutic measures aimed at interfering with these interactions. This project focuses on the adhesive function of 987P fimbriae. These fimbriae are hair-like appendages on the surface of enterotoxigenic *Escherichia coli* . By mediating bacterial binding to the intestines of piglets, the fimbriae promote colonization of the intestinal surface and optimize toxin delivery to the epithelial cells, resulting in diarrhea. A minor 987P fimbrial subunit, designated FasG, mediates bacterial binding to two types of intestinal receptors, namely a set of specific glycoprotein receptors and the glycolipid sulfatide. The aim of this project is to delineate the binding interactions of FasG with its two receptors by using various mutagenesis approaches. To better understand the host's involvement in the disease process, the porcine glycoprotein receptor will be studied by separation and cloning methods. To determine the relative importance of each binding interaction in pathogenicity, neonatal piglets will be infected orally with bacterial mutants unable to bind either to one or to both receptors. By shedding light on the mechanisms of infection of this microbial pathogen of veterinary relevance, this research will guide the design of novel and improved approaches to combat infectious diseases.

9902315 Disease Resistance Lowered by Dissolved Oxygen and pH in Aquacultured Shrimp

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Strengthening Grant; Grant 99-35204-8555; $155,000; 2 Years

Dissolved oxygen has been widely used as a measure of water quality and has been useful in protecting animals and their habitats. The deleterious effects of acute exposure to low oxygen (hypoxia) on shrimp survival are well known. However, little is known of how chronic or periodic hypoxia and elevated water CO_2 (hypercapnia; a concomitant with low oxygen) alter the resistance of juvenile and post-larval shrimp to pathogenic infections of bacteria, viruses, and fungi. In commercial production, shrimp are stocked at high densities and frequently subjected to low oxygen stress. Preliminary data suggest that hypoxia greatly reduces the resistance of shrimp to bacterial infections. We will use an experimental model to assess the influence of hypoxia on the ability of juvenile and post-larval shrimp to resist infection with a common opportunistic pathogen under typical aquaculture situations. We will identify the duration and the intensity of low oxygen that places production stocks at risk for massive losses from bacterial disease. The shrimp, *Penaeus vannamei*, held at non-lethal levels of hypoxia will be exposed to the bacterial pathogen, *Vibrio parahaemolyticus*, and their survival quantified. The results of these studies will establish oxygen levels critical to maintaining resistance against opportunistic infections in shrimp.

9902141 Veterinary Virology Symposium: Comparative Mechanisms of Viral Pathogenicity and Immunity
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Grant 99-35204-7862; $5,500; 1 Year

This proposal requests funds for the First Veterinary Virology Symposium on "Comparative Mechanisms of viral Pathogenicity and Immunity". Viruses are a major cause of morbidity and mortality in food and companion animals. The major economic impact of virus infections include poor growth, loss of reproductive capacity, cost of treating secondary bacterial infections, death of the animal and emotional suffering of producers (result of income loss) and pet owners (loss of companion). In addition, new viruses and viral diseases continue to emerge. Examples of emerging viral diseases include porcine reproductive and respiratory syndrome, bovine virus diarrhea virus type 2, bovine respiratory coronavirus, porcine respiratory coronavirus, avian H1N1 influenza viruses in pigs, canine parvovirus type 2 and circovirus, the putative agent of porcine postweaning multisystemic wasting syndrome. One of the most effective means to bring experts in different disciplines together is through a conference or symposium format. This format allows scientists, veterinary virologists, to acquire new knowledge from colleagues, exchange ideas, compare research data and foster new collaborations in research. Comparative studies of viruses will in the long-term result in novel approaches for the control and elimination of viral diseases. This collaborative approach to the study of animal viruses may result in: 1) treatments, management protocols or control methods that improve the health and well-being of food and companion animals; 2) an improved income for food animal producers; 3) a safer food supply; and 4) the emotional well-being of companion animal owners.

9902296 Receptor Binding Specificity of the K88 Fimbriae of *Escherichia coli*
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Grant 99-35204-7710; $210,000; 3 Years

Colibacillosis caused by strains of *E. coli* that produce K88 fimbriae and infect the small intestines is a common and frequently fatal disease of pigs. However, some pigs are inherently resistant to this condition. Resistance, inherited as an autosomal recessive trait, appears to be in consequence of the inability of the bacterium to adhere via K88 to intestinal epithelium. Developing a practical means of identifying resistant pigs so they can be selectively bred is a long-term goal of our research. Recently, we identified an intestinal glycoprotein (sugar-containing protein) called IMTGP in pigs susceptible to K88+ *E. coli* and to which K88 adheres. We further found that piglets producing IMTGP develop severe diarrhea when inoculated with K88+ *E. coli* strains, while piglets not producing IMTGP do not. This correlation suggests, but does not prove that the *E. coli* causes disease by first attaching to IMTGP. In research funded by this grant, we will determine which amino acids in K88 are critical to its binding of IMTGP. Using molecular techniques, we will also modify the structure of K88 so it not longer binds IMTGP, but is still capable of binding another pig epithelial molecule called IGLad. Bacteria producing the modified K88 will be tested for virulence in pigs. Loss of virulence concomitant with loss of ability to attach to IMTGP will indicate that IMTGP is important to disease susceptibility. Such a finding will focus our efforts on identification of the genes responsible for IMTGP.

9902307 Cellular Trafficking, Intracellular Survival, and Virulence of *Brucella abortus*
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Grant 99-35204-7750; $200,000; 3 Years

Intracellular pathogens take advantage of cellular trafficking pathways or subvert them in order to obtain hospitable environments. In their seminal work, Pizarro-Cerda, et al revealed a significant difference in cellular trafficking among *Brucella* strains. Virulent *Brucella* strains were targeted to LAMP (lysosome associated membrane protein) containing vacuoles, while vacuoles containing S19, an attenuated vaccine strain, contained LAMP and the degradative enzyme cathepsin D. It has been suggested that the inability of S19 to block fusion with degradative vacuoles results in its attenuated phenotype. The drawback of these exciting studies was the use of Vero and HeLa cells, and the genetically undefined S19. Chronic infection with *Brucella* is attributed to intracellular survival within macrophages and directly correlates with virulence in the primary host. In these studies we propose the use of bovine monocyte derived macrophages to characterize the maturation of the *Brucella* containing phagosome, and the genetic characterization of mutants identified by their failure to survive in mice and their inability to survive intracellularly. In addition, we propose an examination of the maturation and cellular trafficking of *Brucella* containing vacuoles using immunofluorescent staining and confocal microscopy. These studies will provide preliminary results on the suitability of identified proteins as targets for anti-*Brucella* vaccines designed to prevent colonization of cattle or the use of the attenuated mutants directly as improved vaccine strains.

9902310 Regulation of *Pasteurella haemolytica* Leukotoxin Expression

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Grant 99-35204-7875; $262,500; 3 Years

Bovine respiratory disease complex (BRDC) is a severe respiratory disease that costs the United States cattle industry nearly a billion dollars per year. The primary bacterial agent involved in BRDC is *Pasteurella haemolytica*. Paradoxically, *Pasteurella haemolytica* is part of the normal flora of the upper respiratory tract of healthy cattle. Under conditions of stress and immunocompromise, the bacterium can become pathogenic. Emergence of widespread antibiotic resistance and the lack of effective vaccines have prevented control of this disease. The major virulence factor of *P. haemolytica* is a leukotoxin that kills white blood cells. We hypothesize that expression of the toxin responds to environmental cues within the bovine respiratory tract. These cues activate leukotoxin production and likely activate expression of other virulence genes, thereby causing the switch to the pathogenic state. We have shown that one of these cues is a shift to internal body temperature, or 37°C. We will use a genetic reporter system to study the DNA and protein elements that are responsible for thermomodulation of leukotoxin expression. We will also measure leukotoxin expression under other conditions that may modulate its expression and will look for other genes that are similarly regulated. Our goal is to understand the environmental cues that make the *Pasteurella haemolytica* organism switch from benign flora to lethal pathogen. If the mechanisms of this signal can be deduced, it may be possible to design strategies to prevent it.

9901980 Molecular Basis of LOS Phase Variation and Sialylation in *Haemophilus somnus*

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Grant 99-35204-7670; $240,000; 3 Years

Haemophilus somnus is the cause of various diseases of cattle, including pneumonia (shipping fever), neurologic disease, abortion, septicemia, arthritis, and others. Due to this wide variety of diseases, *H. somnus* may be the most economically important pathogen of feedlot cattle. Currently available killed vaccines are inadequate, and improved vaccines need to be developed through more thorough understanding of the interaction between the bacterium and the host. *H. somnus* is able to change the carbohydrate structure on its surface [lipooligosaccharide (LOS)] through a process called phase variation. Phase variation occurs due to repeating sequences of 1, 2 or 4 DNA nucleotides that occur between where the gene is first read and the part of the gene that encodes for protein. When the number of these repeating sequences change during replication, the gene can be turned on or off. We have identified two genes in *H. somnus* that are involved in LOS synthesis and contain such repeats. Inactivation of one of these genes reduced, but did not eliminate, the phase variation rate and virulence of *H. somnus*. Our objectives for this project are to identify additional genes responsible for LOS synthesis, phase variation, and attachment of sialic acid. We will make mutations in these genes to inactivate them and make a series of mutants containing various truncations in its LOS. Such mutants will enable us to better understand how these genes and LOS enable the bacterium to cause disease. Our goal is to use this understanding to develop improved vaccines to prevent *H. somnus* diseases.

9902304 Effects of Vertical Infections of *Renibacterium salmoninarum* on the Salmon Immune Response

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Postdoctoral Fellowship; Grant 99-35204-8587; $90,000; 2 Years

Renibacterium salmoninarum (Rs), causative agent of bacterial kidney disease (BKD), is one of the most important pathogens of wild and cultured salmonid fishes worldwide. The pathogen causes systemic infection that can be transmitted both horizontally (fish to fish), and vertically (parent to progeny). There is no effective way to control the disease using antibiotics or vaccines. The potential for the development of immunological tolerance to Rs due to vertical transmission is a variable that has not been adequately investigated. Salmon fry infected with Rs as unfertilized eggs may be unable to distinguish important protective Rs immunogens from self proteins, leaving them unresponsive to vaccination. The purpose of this research is to determine the effect of vertical transmission of viable Rs cells on the development of the salmon immune response to vaccination and challenge with Rs compared to controls. Specific objectives are to determine if vertical infection affects vaccination efficacy or antibody and macrophage responses, and if the effects are due to a generalized immunosuppression or specific immunological tolerance to Rs antigens. This research will increase knowledge needed to prevent or reduce severity of disease in salmonids by providing information necessary for development of more effective immunization strategies for BKD. The results could also be relevant to other vertically transmitted pathogens in salmon as well as other aquaculture species.

9902451 *In Vivo* Model Using *Escherichia coli* DNA as an Adjuvant to Induce Type 1 Cytokine and Antibody Responses

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Grant 99-35204-8368; $300,000; 3 Years

The technology of DNA-based immunization has revolutionized vaccine development, and is especially applicable for use in domestic animals to immunize against a variety of viral, bacterial, and parasitic pathogens. One reason DNA vaccines are so effective in mice is the adjuvant effect of the DNA plasmid on host antigen presenting cells (APC), including the primary cell used during immunization, the dendritic cell. Specifically, *E. coli* DNA and immunostimulatory motifs in non-vertebrate DNA induce cytokines, such as interleukin-12 (IL-12) in APC, that stimulate strong cell-mediated immunity. Before DNA vaccines become marketed for cattle, it is essential to understand how bacterial DNA modulates the bovine immune response. This proposal will examine cytokine induction in dendritic cells by bacterial DNA and will compare the use of *E. coli* DNA and IL-12, alone or in combination, as adjuvants to prime for enhanced cell-mediated and antibody (IgG) responses in cattle immunized with an important major surface protein of *Anaplasma marginale*. *A. marginale* is an economically important cattle pathogen in the United States, for which a vaccine is needed. Both cellular and humoral immune responses are hypothesized to be critical for protective immunity to this ehrlichial pathogen. The efficacy of the immunization regimens will be determined following challenge infection. This project will provide novel and basic information on the immunological consequences of using *E. coli* DNA as an adjuvant in cattle, which can be extended to the use of DNA vaccines.

9902050 Effect of Antigen Modification on the Immune Response to Candidate Synthetic Vaccines

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Grant 99-35204-8556; $187,685; 2 Years

Efforts to develop effective vaccines to blood parasites have shown that synthetic and recombinant vaccines obtained from these parasites have not been as effective in eliciting protection as crude membrane fractions or whole live organisms. It is possible that the synthetic vaccines are not being presented in a form that elicits the appropriate immune response. Antibody plays a protective role with some parasites while cell associated immunity is more important with other parasites. The candidate vaccines to blood parasites in the past have elicited strong antibody responses that have not correlated well with protection. It may be that cell associated immunity is a critical component. To examine this possibility, we will develop an *in vitro* model in cattle that will allow us to manipulate the immune response to an *Anaplasma marginale* peptide antigen. The initial studies will focus on analysis of the effect of coupling the sugar mannan with the peptide under oxidizing and reducing conditions on the cytokine profile of antigen presenting cells. Coupled antigen under these conditions has been shown to change a nonprotective antibody response to a protective cellular immune response in a mouse model system. If mannan has the same effect in cattle, we will have a system to test the effect of antigen modification on the immune response elicited by modified candidate synthetic vaccines and correlate specific changes in vaccine composition with the capacity of the vaccine to elicit protection.

9902054 Enhancing Antigen Presentation for DNA Vaccines

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Grant 99-35204-8274; $190,000; 3 Years

DNA vaccines offer advantages over many existing vaccines. Consequently, DNA vaccines against a wide variety of microbial pathogens of livestock are currently under development. Testing of these vaccines has revealed several problems that limit their widespread use in livestock. These include the need for multiple immunizations using a large dose of vaccine per immunization to induce immunity and the significant variation in the response among individual animals. The percentage of vaccinated animals that respond with weak immune responses is exacerbated when either low or fewer doses of vaccine are used. This indicates that the problem of weak response is, in large part, attributable to inefficient processing of the vaccine by the immune system. Our goal is to improve DNA vaccines for cattle by enhancing the requisite vaccine processing pathway, termed "antigen presentation". Dendritic cells are the essential antigen-presenting cells. The proposed research will utilize a specific inducer of dendritic cells, flt3 ligand, to enhance dendritic cell recruitment to the site for DNA vaccine immunization. A second factor, granulocyte-macrophage colony stimulating factor (GM-CSF) will be included to enhance complete dendritic cell maturation and activation. These two bovine immune system factors will be included in a DNA vaccine containing the *Anaplasma marginale* MSP1 (a surface protein that is a component of effective vaccines against bovine anaplasmosis). It will be determined if they enhance recruitment and maturation of bovine dendritic cells and improve vaccine efficacy. The results will be applicable to DNA vaccines developed against any pathogen of cattle.

9902072 Tegument Protein VP22 Facilitates Bovine Herpesvirus Infection

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Grant 99-35204-7933; $200,000; 2 Years

Of the three major structures that make up bovine herpesvirus virions-the capsid, tegument, and envelope- the tegument is the least understood. Located between the capsid and envelope, the tegument consists of proteins critical to viral survival and appears to subjugate host cell function. One of the most prevalent tegument proteins is termed VP22. In preliminary studies, VP22 possesses the unusual property of binding to the cell cytoskeleton and nucleus. Also, in preliminary studies, VP22 protein produced from one transfected cell traffics to neighboring non-transfected cells. The most stimulating bovine herpesvirus vaccine appears to require VP22. Therefore, an ideal BHV-1 vaccine would likely contain a sufficiently mutated VP22 to cripple virus replication yet stimulate a potent immune response. Now, using plasmids expressing VP22 and mutant viruses, we will elucidate the mechanisms of VP22 to viral infection by the following objectives: 1) to determine how VP22 interacts with host cells, segments of VP22 important in cytoplasmic localization, nuclear association and cell-to-cell trafficking will be determined by deletion mutants of VP22; 2) to determine the role of VP22 in BHV-1 infection *in vitro*, virus mutants will be constructed. The amount of infectious virus produced by viral mutants will be compared to wild type virus and the replication impaired BHV-VP22 gene deletion virus. Thus, we will determine the importance of specific VP22 motifs for virus replication. In summary, we will determine the importance of these newly identified regions of VP22 in virus replication by engineering altered viruses. Identifying these fundamental VP22 motifs will be useful in vaccine strategies where VP22 is a potent stimulus to T cell immunity. These specific objectives fill an important gap in defining the mechanisms of a tegument protein in BHV-1 pathogenesis.

PEST BIOLOGY AND MANAGEMENT

The broad goal of this topic area is to support fundamental and mission-linked research on the biology of pest species, the agents that suppress pests, and the interactions between plants and their pests. Grants are awarded within four program areas: Biologically Based Pest Management, Entomology and Nematology, Plant Pathology, and Weed Biology and Management.

PLANT PATHOLOGY

Panel Manager - Dr. Margaret E. Daub, North Carolina State University
Program Director - Dr. Ann Lichens-Park

Research grants in this program support studies on stress on plants arising from their interactions with microbial agents including fungi, bacteria, viruses, and mycoplasma-like organisms. The ultimate goal is to limit losses in plant productivity from damage caused by biologically-generated stresses.

Emphasis in this area is on studies that will enhance understanding of how stressful interactions are established between plants and pathogens; how such interactions are influenced by environment and other factors inherent to the interacting organisms; how the interactions reduce plant productivity and usefulness to people; how plants react to stress generated by such interactions; and how the damage of such interactions may be limited or eliminated.

9902572 Function of Root Border Cells in Plant Health: Pioneers in the Rhizosphere
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Grant 99-35303-8140; $140,000; 2 Years

Plants of most agronomically important species can produce thousands of healthy single cells, root "border" cells, that are programmed to separate from their root tips into the soil. The function of this unusual and costly process is unknown, but properties of the cells are consistent with the hypothesis that they protect plant health by forming a protective layer (border) around the root tip, enabling the root to dictate which microorganisms can share its ecological niche. Previously, we have described a major new regulatory network that controls the production of border cells, identified several genes that play a role in the process, and defined endogenous and environmental signals that control their expression. These tools have been used to demonstrate that the properties of the rhizosphere can be altered drastically in response to normal stimuli facing roots, and that such changes can significantly alter the susceptibility of roots to infection by at least one soil borne fungal pathogen. The goal of this renewal application is to determine the impact of living root border cells on the ability of plants to develop relationships with agriculturally important organisms including nematodes, bacteria, and fungi. Transgenic roots with non-living border cells, will be employed to examine the hypothesis that a defined change in border cells will alter the ability of pathogenic and symbiotic organisms to colonize and/or infect plant roots, and to initiate studies to determine the mechanism(s) by which border cells influence root infection. Understanding how border cells work will increase the chances that they can be used to develop environmentally sensitive new methods for disease control, and provide a framework to predict the kinds of ecological changes that may result from the genetic engineering of border cells.

9902566 Tolerance to Phytoalexins: a Host-Specific Virulence Trait Shared by Different Pathogens?
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Grant 99-35303-8681; $117,815; 2 Years

The aim of this proposal is to understand how resistance to plant antibiotics (phytoalexins) is acquired by plant pathogenic fungi and whether it allows these fungi to overcome mechanisms of plant disease resistance that are based on phytoalexin production. The experimental system consists of pea *(Pisum sativum),* its phytoalexin pisatin and fungal pathogens of pea. The pea pathogen *Nectria haematococca* can detoxify pisatin through the action of specific enzyme called pisatin demethylase (pda) and previous studies have shown that pda functions as a virulence factor for this fungus. N. *haematococca* also possesses another means to avoid the toxic effects of pisatin, which appears to involve the ability to change the entry rate of the compound into the fungus. This tolerance mechanism has been called a "non-degradative" tolerance mechanism. We will create mutants that lack both pda and non-degradative tolerance in order o determine if non-degradative tolerance is also a virulence factor and works in concert with pda to allow this pea pathogen to avoid the inhibitory effects of pisatin. Completion of this objective will reveal whether this pathogen uses complementing tolerance mechanisms to overcome a plant defense mechanism based on phytoalexin production. Having such information should help in creating new resistance mechanisms or altering the normal in plants so that they have long term durability.

9902479 Significance of Aggregation in Epiphytic Bacterial Populations
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Grant 99-35303-8633; $155,514; 2 Years

Plants are normally colonized by large numbers of epiphytic bacteria which can either be beneficial or detrimental to plant growth. Knowledge of factors that contribute to the ability of bacteria to live on leaves is important in devising strategies of biological control of disease and to understand how disease is initiated by bacteria on plants. While epiphytes can occur as solitary cells, the majority of the population often occurs relatively large aggregates and the environment within

such aggregates is apparently different from that of solitary cells and contributes to the survival of stresses on leaves, and successful immigration to a leaf. We will study the development of aggregates on leaves and their role in survival and proliferation of bacteria. We also will determine the spatial heterogeneity of cells to determine if interactions are minimized due to separation of different bacterial species. The viability of cells of different aggregation states will be measured under different environmental conditions using nucleic acid stains that are differentially permeable to living cells. The survival and proliferation of immigrant cells of a green fluorescent protein (GFP)-marked bacterial strain will be studied on leaves by visualizing cells exhibiting GFP fluorescence. The chemical environment and the physiological status of cells within aggregates will be assessed using whole cell biosensors harboring a GFP reporter gene fusion to promoters responsive to fructose or reflective of the growth rate, respectively. Reporter gene activity will be determined by quantification of the fluorescence of individual cells by analysis of digitized microscopic images.

9902484 Seventh International Symposium on the Microbiology of Aerial Plant Surfaces
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Grant 99-35303-8639; $5,775; 1 Year

Research on the microbiology of the phyllosphere has many significant implications for successful crop production and human health worldwide since plants support diverse populations of plant and human pathogenic microorganisms. An understanding of the interactions that occur among phyllosphere microbes and between these microbes and the host plants that they colonize will enable better disease control. Basic aspects of microbial ecology such as gene exchange, habitat modification, and interactions of microbes with the hosts on which they live are also often best examined in this system. To obtain this understanding, communication among scientists from diverse disciplines must be encouraged and fostered. Funds will support the Seventh International Symposium on the Microbiology of Aerial Plant Surfaces, to be held in Berkeley, California from August 3-8, 2000. Objectives of the symposium are: 1. Provide a forum for review and critique of the major findings related to the microbiology of the phyllosphere from 1995 to 2000. 2. Provide a forum in which research in the microbiology of the phyllosphere can be discussed in relation to developments in other areas of microbial ecology, and an arena in which new and current ideas and concepts can be discussed and criticized in this context. 3. Stimulate discussion and speculation as to where future phyllosphere research efforts may encounter the greatest potential gain. 4. Produce a published volume that summarizes progress in the field over the past five years, describes current thinking in the area, and leads toward future progress. 5. Stimulate research in all aspects of the microbiology of the phyllosphere.

9902498 Molecular Determinants of Crinivurus Whitefly Transmission
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Grant 99-35303-8634; $225,000; 3 Years

Plant viruses are important impediments to productive agriculture, ant several plant viruses affect the plants we grow for food and fiber. A newly emerging group of important plant viruses is represented by lettuce infectious yellows virus (LIYV), type member of the genus *Crinivirus.* LIYV is specifically transmitted from plant-to-plant by the sweet potato whitefly, *Bemisia tabaci.* This proposal is focused towards understanding LIYV-encoded molecular determinants facilitating B. *tabaci* transmission of LIYV. Initial efforts will be directed towards identifying LIYV-encoded proteins that determine formation of LIYV virus particles. Virus particles are what are transmitted from plant to plant so understanding the events and determinants in their formation is essential. We will create specific mutations in LIYV genes and assess effects on virus particle formation within LIYV infected cells. When mutants are characterized we will attempt to restore function by *in bans* complementation using virus transient expression vectors. Next, we will focus on identification of LIYV-encoded proteins which affect the ability of LIYV virions to be transmitted by *B. tabaci.* We will I use the mutants identified previously and use specific molecular markers to assess transmission of LIYV to plants. Finally, we will focus on one of the LIYV virus particle proteins' the minor capsid protein (Cpm). A series of substitution mutations will be generated to determine effects of specific protein domaine on LIYV virion formation and whitefly transmissibility.

9900574 Delivery of Pathogen Signals to Plants
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Grant 99-35303-8055; $2,000; 1 Year

Plant-Pathogen interactions has been one of the most productive research areas in all of biology in the last several years. Important new information has been obtained on virulence functions in plant pathogens and how these virulence factors are delivered to plant cells. Functions for avirulence gene products are being deduced and many plant disease resistance genes have been cloned and characterized. Of particular interest, the leucine rich repeat domains of these proteins have been shown to convey the specificity for recognition of pathogen elicitors, although it is not yet clear that they are

receptors for the elicitors. Progress is also being made in transforming cloned disease resistance genes into new plant species and evaluating them for disease resistance. Finally, progress is occurring in both biochemical studies and mutant hunts for components of intracellular signaling pathways that are involved in active plant disease defense. All of these topics will be discussed by world leaders in their respective fields at the 8th Japan-US Seminar.

9902410 Mechanism of Coat Protein-RNA Interactions in Bromoviruses

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Grant 99-35303-8155; $225,000; 3 Years

Knowledge regarding how viral coat proteins interact with their genomic RNAs is valuable in understanding the mechanism of assembly and disassembly of infectious virions. Successful assembly process into infectious and stable virions is dependent on the extent of interaction between the viral coat protein (CP) and its genomic RNA. The overall goal of the proposed project is to define and characterize the important contact sites between coat protein-RNA in multicomponent plant RNA viruses. Using brome mosaic virus (BMV) as a model system, we will analyze the capsid dynamics, molecular and genetic aspects of selected BMV coat protein variants having unique terminal arms and exhibiting altered pathogenesis on a wide spectrum of host plants. A variety of molecular techniques will be employed to identify critical interaction sites distributed on a single CP and multiple RNA sequences that actively participate in virion assembly prior to establishing infection in susceptible host plants. The proposed studies will have fundamental importance in providing a better understanding of how viral coat proteins interact with their genomic RNAs during virion assembly and cause disease in agronomically important crop plants. Each proposed objective addresses molecular interactions at increasingly complex biological levels with the promise of being able to apply what is learned about RNA-coat protein interactions to understanding the overall infection process. Most importantly, results obtained from the proposed studies will have broad application to many other viral systems and likely to help in devising novel strategies of virus disease control to reduce losses in economically important crop plants.

9902527 Genetic Analysis of the Ustilago maydis-Maize Interaction.

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Grant 99-35303-8635; $130,000; 2 Years

Corn smut is a plant disease of cultivated maize. The fungus that causes this disease is a close relative of other pathogens of many grain crops. The corn smut fungus and its relatives can grow in two forms. The first form is a yeast with small dividing cells. The second form is typical of most fungi and is made up of long tubular cells or filaments. In corn smut the yeast form cannot attack the plant but the filaments can. These two forms are also common in animal and human pathogens and their form is often correlated with the ability to cause disease. We have discovered genes that control the ability of the fungus to grow in these two forms. We will employ a strain that is permanently fixed in the filamentous morphology to identify genes specifically expressed in the filamentous or in the budding morphology. Additionally, we will use another mutant fixed in the budding morphology as a unique control organism to identify fungal and plant genes specifically expressed in gall formation. We will disrupt two fungal genes identified in each screen and we will determine the phenotypes of the resulting mutant strains with respect to morphology and pathogenesis. This information will have wide significance for understanding fungal growth and disease in general. It may provide new insights into novel targets for plant disease control. The results will likely have important implications for animal and human fungal diseases as well.

9902588 Role of Plant-Bacterial Surface Interactions in Epiphytic Colonization

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Grant 99-35303-8301; $165,000; 3 Years

The goal of this proposal is to identify how specific surface properties of plants and bacteria influence the growth and survival of bacteria on leaves. Because the major point of contact between leaf surface bacterial populations and the plant host is at the waxy layer called the cuticle, the cuticle is likely to be a dominant plant trait influencing the leaf surface as a habitat for bacteria. This proposal will examine how this seemingly inhospitable waxy surface and bacterial cell surfaces interact to influence distinct colonization processes. The objectives of the proposal are 1) to evaluate how various features of plant cuticles influence the ability of bacteria to grow, survive, and enter into leaves, and 2) to evaluate how changing a major bacterial surface trait, namely the presence of extracellular polysaccharides, affects the relationship between cuticles and leaf colonization.

An understanding of the factors that influence bacterial colonization of leaves should contribute to effective strategies for reducing disease losses. Because large populations of plant-pathogenic bacteria on plant leaves are often associated with a high incidence of disease, reducing their populations can reduce disease. The results of these studies will

provide targets in plants and bacteria for hindering colonization by plant pathogens or for fostering colonization by biocontrol agents. They will also improve our ability to predict foliar diseases and biocontrol effectiveness by indicating how bacterial populations are influenced by changes in plant cuticles. Such cuticle changes may result from air pollution, water stress, pesticides, or genetic engineering of plants for enhanced drought resistance.

9902580 Role of a Fungal Extracellular Matrix in Lesion Development

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Grant 99-35303-8139; $160,000; 2 Years

The overall goal of this research is to understand how fungi cause plant disease. In particular, we want to test the importance of the slime layers that surround many fungi. These slime layers form the closest point of contact between such fungi and their plant hosts; thus, they have the potential to perform a variety of functions important in disease, especially once the fungus is inside the plant.

To test whether fungal slimes are important inside plants, we will clone a gene from the fungus *Cochliobolus heterostrophus*, causal agent of southern corn leaf blight. This gene encodes a slimy, mucilaginous layer that surrounds the fungus and is dispensable when the fungus is on the surface of corn leaves, but appears to be essential when it is inside. We will then use the cloned gene to disrupt the resident gene in a wild-type strain. If the disrupted strain loses the ability to cause disease, then the slime layer was needed inside the plant. We will use microscopy to confirm the presence/absence within the plant of the slime layer around the wild-type/mutant strains, respectively.

If this research demonstrates that fungi need slimes when they are within plant tissues in order to cause disease, then it may be possible to control at least some plant diseases by engineering plants to degrade the slime material.

9902523 The Spectra of Variants in Potyvirus Populations

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Strengthening Award; Grant 99-35303-8632; $100,000; 2 Years

The existence of RNA viruses as quasispecies - a mixture of viral sequences containing a predominant sequence, and a variable mixture of related but molecularly distinct sequences - has observed for a number of mammalian and bacterial viruses. These viruses appear to evolve at a very rapid rate because of the lack of proofreading of RNA-dependent RNA polymerases. Thus, these important disease agents can mutate at rates that are on the order of size magnitudes greater than DNA-based organisms. However, little data are available for the plant viruses. This proposal is aimed at obtaining information on sequence diversity in certain populations of legume-infecting potyviruses, and determining the nucleotide substitution frequencies in these populations. The extent and nature of nucleotide sequence variants in populations of certain legume-infecting potyviruses will be examined. As these populations are characterized, we hope to then determine how and at what rate population structure may shift, over time, in response to selective pressures, such as host plant resistance. Understanding the nature of potyvirus evolution will provide insights as to how these viruses may respond to various environmental and selective factors in nature. Ultimately, we distributions of molecular sequences and its fitness. Since these are among the most economically important viruses, this information may facilitate classical and biotechnological breeding for virus resistence in crop

9902503 Mechanism of Viral Subgenomic RNA Synthesis II

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Grant 99-35303-8091; $220,000; 3 Years

Viruses are important pathogens of crops, humans and livestock. Effective strategies to stop viral infections rely on basic knowledge of the replication mechanism of viruses. Our studies focus on a class of viruses that contain ribonucleic acids (RNAs) as their genetic material, with emphasis on a model pathogen of cereal crops, including wheat and corn, named brome mosaic virus. Using in vitro assays, we *have* defined the requirements in the BMV RNAs needed for successful replication. In this work we seek to: 1) define the physical and chemical parameters in the BMV RNAs that directs RNA replication; 2) identify the atoms in the substrate nucleotide, GTP, needed to start RNA synthesis; 3) define the enzymatic requirements in the proteins needed to direct RNA replication. Our USDA funded work has already led to the submission of a patent application on inhibiting viral replication and another to detect viral RNAs. Further work should reveal additional methods to prevent and diagnose infections by RNA viruses.

9902511 Surface Architecture of Cucumber Mosaic Virus and Aphid Vector Transmission

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Grant 99-35303-8638; $165,000; 3 Years

The control plant virus diseases will be a significant component of long-range improvement in and sustainability of U.S. agriculture. Virus diseases are largely controlled by prevention and/or host resistance, and avoiding or reducing vector activities is an important component of crop management. Most plant viruses have vectors, most plant virus vectors are insects, and of these a majority are aphids. Cucumber mosaic virus and a large number of other plant viruses are transmitted non-persistently by aphids; this is the most common type of vector transmission for known plant viruses.

An attractive means of disease control is to interfere with the ability of an aphid to acquire and transmit virus. Characterizing the surface architecture of the virus is a prelude to understanding mechanisms of vector transmission and to identifying how virions interact with the aphid. This will lay the groundwork for the development of novel virus disease control strategies which target the transmission process.

The long-term goal of this research is to understand the relationship between the structure of cucumber mosaic virus and the process of virus transmission by an aphid vector. The specific objectives of this project are: 1) To characterize structural (e.g. coat protein) viral determinants of transmission, 2) To evaluate the role of virion stability in transmission, 3) To identify candidate virus receptors in the aphid, and 4) To determine if virus mutants which are defective in aphid transmission are able to bind in the aphid styles.

9902509 Identification of Pathogenicity Mutants of *Colletotrichum graminicola*

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Postdoctoral Fellowship; Grant 99-35303-8191; $90,000; 2 Years

Colletotrichum graminicola is the causal agent of anthracnose leaf blight and anthracnose stalk rot of corn. While this fungus has been recognized as a pathogen of corn in the U.S. since the 1960s, there has recently been an increase in the prevalence of this disease, possibly due to changing agricultural practices. The goal of this project is to identify genes from C. *graminicola* that are important for pathogenicity to corn leaves. A procedure known as insertional mutagenesis will be used to create fungal strains (transformants) that contain randomly disrupted genes. This procedure simultaneously disrupts and tags genes with a short piece of DNA. Transformants will be tested on corn seedlings in order to identify those with a disrupted pathogenicity gene. Since the disrupted genes are tagged, they can be easily isolated for subsequent study of function. The objectives of this project are: 1) perform tests of transformants to identify those with increased or decreased levels of aggressiveness to corn seedlings and 2)characterize the mutants found in step one through classical and molecular genetic analysis in order to identify the gene.

9902519 Functions of the Tobamovirus Replicase Implications in Replicase-Mediated Resistance

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Grant 99-35303-8637; $149,000; 2 Years

Genome replication is one of the most fundamental processes in biology. For positive-stranded RNA viruses, an essential aspect of this process is provided by viral encoded replicase genes. In general, these replicase genes encode a number of functional domains that participate in sequence-specific RNA synthesis, virus transport, and disease development. Unfortunately, our knowledge of these essential virus genes, including their functions and interactions, is at best poor. In addition, the mechanisms underlying the ability of specific replicase domains to confer resistance against subsequent virus infections in both transgenic and classical cross-protection systems remains unclear. The objectives of this proposal are to utilize a defined tobamovirus system to investigate and characterize the ability of different replicase domains to confer resistance. Specifically, a heterologous virus vector system will be used to individually test domains of the tobacco mosaic tobamovirus replicase gene for their ability to bestow resistance. Domains that confer resistance will be further examined to identify the sequences and motifs that are responsible for the resistance. Additional studies will be directed at determining the mechanisms behind the ability of specific replicase domains to block virus infections. Information from this study should provide insight into both the functional aspects of viral replicase genes as well as new methods to manipulate and expand the usefulness of replicase genes as antiviral agents.

9902420 *Pseudomonas syringae pv. tomato* Hrp-controlled Extracellular Proteins

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Grant 99-35303-8008; $225,000; 3 Years

The long-term goal of this research is to increase our understanding of the molecular mechanisms of bacterial pathogenesis in plants. A good understanding of bacterial pathogenicity, together with increased knowledge of plant disease resistance mechanisms, provide critical information for the design of novel, environmentally safe strategies for disease control. Research in the past decade has clearly shown that hypersensitive reaction and pathogenicity *(tarp)* genes play a central role in the pathogenesis of major Gram-negative bacterial pathogens, including *Pseudomonas syringae.* A major function of *hrp* genes appears to be the assembly of a type III protein secretion pathway (the Hrp pathway) for delivery of putative virulence proteins into plant cells. However, the actual mechanism of *tarp-mediated type HI* protein secretion *and* the effects of *tarp-controlled* virulence proteins on host signaling or metabolic pathways are elusive. In P. *syringae py. tomato* DC3000 *(Pst* DC3000), which infects *tomato and Arabidopsis, hrp* genes *are* involved in *the* assembly of a novel surface appendage called the *Hrp* pilus. The assembly of *the* Hrp pilus appears *to* be essential for *plant-Pst* DC3000 interactions. In *this* research, we will *attempt to reveal the* functions of *the* Hrp pilus. Specifically, we will characterize the effect of pilus mutations on *the* regulation and secretion of *Pst* DC3000 virulence proteins. The following specific objectives will be pursued: 1) Determination of the molecular basis of the requirement of the Hrp pilus structural protein, HrpA, for *plant-Pst* DC3000 interactions and 2) Characterization of HrpA interaction with plant cell walls.

9902414 Co-evolution of Phytotoxin Production and Auto-Immunity in a Pathogenic Fungus

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Grant 99-35303-8000; $218,000; 3 Years

Filamentous fungi are the leading cause of plant disease loss in the United States. A better understanding of the mechanisms by which plant pathogens attack plants is necessary for the development of more efficacious and environmentally benign strategies to control plant diseases. Fungi in the genus *Cochliobolus* have caused serious crop losses of cereals in the United States. Many species of *Cochliobolus* make low-molecular weight molecules, called host-selective toxins, which allow them to pathogenize plants. We are studying the mechanism by which *Cochliobolus carbonum,* a pathogen of com, protects itself against its own host-selective toxin. The target site of the toxin is an enzyme called histone deacetylase, which is present in all higher organisms including plants and mammals. We have discovered that the histone deacetylase of *C. carbonum* itself is uniquely resistant, among all known histone deacetylases, to its toxin. This presumably serves to prevent the fungus from killing itself. An understanding of the biochemical mechanism of this resistance is the basis of the proposed research. We will determine if resistance is an inherent property of the *C. carbonum* enzyme or if resistance is due to some other factor. If resistance is inherent, we will transfer the fungal gene encoding the resistant enzyme into com to see if this will confer resistance to the toxin and hence to the pathogen. The results will contribute to a better understanding of the molecular adaptations that have evolved in fungi that allow them to exploit the ecological niche of plant pathogenesis.

9902589 Disruption of the Sexual Cycle of Fungal Plant Pathogens with Pheromone Analogs

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Grant 99-35303-8086; $130,000; 2 Years

This research involves the development of a novel strategy to control fungal plant diseases based on disruption of the pathogens sexual cycle. Fungal sexual spores are often long-term survival structures that act as the primary inoculum to spread disease in tune and space. The sexual cycle increases genetic variability and allows the introduction ant spread of virulence and fungicide resistance genes. Some fungi, including the smut and bunt fungi, must mate to become pathogenic. Mating disruption should allow for the specific, environmentally safe, control of targeted pathogens).

Many fungi locate potential mating partners using chemical signals called pheromones Fungal pheromones are closely related to other important eukaryotic signals, including some involved in cancer. Medical research has focused on using inhibitors to curtail synthesis of these signals. We have found that *Ustilago hordei,* which causes barley covered smut, produces pheromone-related compounds that inhibit mating of *many Ustilago spp.* and teliospore germination of *Tilletia spp.,* all of which must mate to become pathogenic. These pheromone-related chemicals could, therefore, be used to control these diseases. The goals of this project are to 1) determine how the pheromone analogs inhibit U: *hordei* mating, 2) detennine which structural moieties of the analogs are important for activity and 3) alter one of the U. *hordei* pheromone genes so that the cells produce inhibitors instead of pheromone. The ability to design sexual cycle inhibitors of any fungus could provide a broadly applicable, biorational strategy to control fungal diseases.

9902510 Construction of an Ordered, Complete Genomic Library for the X-disease Phytoplasma

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Strengthening Award; Grant 99-35303-854; $100,000; 2 Years

Phytoplasmas are a group of cell wall-less, insect-transmitted prokaryotes. They systemically parasitize plants in phloem tissues and cause diseases in more than 300 species worldwide, resulting in multi-billion dollar losses each year in the U.S. alone. To date, there are no effective measures to control or cure these diseases because nearly all key issues related to phytoplasmas have not been resolved. The objective of this proposal is to construct, the first of its kind, an ordered, single-copied, complete genomic library for the X-disease phytoplasma. We have developed/modified the "Long-Distance-PCR Genome Walking" strategy, a cycle of primer design, PCR amplification, end sequencing, and new primer design. We have "walked" six steps both upstream and down stream from the tRNA"e gene in the 16S/23S rRNA spacer region. The largest amplified fragment in each direction and each step have been saved/cataloged as a part of the genomic library. We will continue "walking" through the genome of the X-disease phytoplasma, thus establishing a single-copied, complete genomic library with known orders. Our long-term goals are to completely sequence the entire genome of the X-disease phytoplasma, and to utilize the sequence data to study critical questions in phytoplasmology, such as culturability, classifications and phylogeny, host/parasite interactions, genetic variation. Better understanding of phytoplasma's evolution and host and vector interactions will eventually lead to developing strategies to control or cure the X-disease and other phytoplasma-associated diseases, thus enhancing the competitiveness of U.S. agriculture /horticulture /forestry in the increasingly competitive world market.

9902490 Characterization of the *Arabidopsis HRTGene,* which Confers Resistance to Turnip Crinkle Virus

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Grant 99-35303-8087; $225,000; 3 Years

Plant disease causes an estimated loss worldwide of $100 Billion annually. One of the most effective ways to prevent disease is through resistance genes. In general, these genes confer on the plant the ability to recognize the attacking pathogen and initiate a complex set of defense responses, which prevent the replication and/or spread of the pathogen. During the past several years, the cloning of a dozen or more resistance genes from a variety of plants (e.g. tobacco, barley, flax, maize, tomato, and *Arabidopsis)* has been reported in the literature. However, only one of these confers resistance to a virus (N gene against tobacco mosaic virus). We have identified and fine mapped another viral resistance gene, *HRT,* from the model plant, *Arabidopsis.* HRT allows *Arabidopsisto* recognize turnip crinkle virus (TCV) and mount a hypersensitive resistance responses (HR) to it. *HRT i*s located on a 40 kb DNA fragment located on the lower arm of chromosome 5.

We will complete the cloning of *HRT* and characterize its structural relationships to other resistance genes as well as pattern of expression. The physical interaction of HRT with the TCV coat protein will also be analyzed since recent data argues that its recognition elicits the HR response. The TCV resistance pathway will also be dissected by identifying cellular protein(s) with which HRT interacts and analyzing the effects on HRT-mediated resistance of *Arabidopsis* mutants which disrupt several transduction pathways. Characterization of *HRT* and its signal transduction pathway should provide important insights into how plants protect themselves against viral pathogens and valuable clues as to how effective disease resistance can be engineered in a wide variety of unrelated crop plants.

9902529 Induction Mechanism of Cell Death and Pathogen Resistance by Bacterioopsin.

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Grant 99-35303-8636; $200,000; 3 Years

Plant diseases caused by viral, bacterial and fungal pathogens can result in severe crop Losses. On the other hand, application of chemicals such as fungicides can be costly economically as well as environmentally. Engineering of broad spectrum disease resistance in crop plants by introduction of foreign genes is an alternative strategy that may alleviate some of these problems. We have discovered several years ago that transgenic expression of the bacterioopsin (bO) gcne, which encodes a membrane protein from rhe bacteria *Halobacterium halobium,* can induce disease resistance behavior in various dicotyledonous species such as tobacco, potato and eggplants. bO induced resistance has been demonstrated to provide protection against certain viral, bacterial, as well as fungal pathogens. Thus, the efficacy of bO induced resistance is not limited to a particular plant species or specific types of pathogens. As such, bO is a good candidate gene tor the ectopic induction of broad spectrum resistance in various crop plants. To this end, we would like to characterize the mechanism through which bO activate plant defenses. Our preliminary work indicated that this protein activates the innate plant defense pathways through its ability to transport protons across membranes. In this current funding period, we will carry out more detailed molecular and biochemical studies designed to localize the site of expression of bO in plant cells and to determine the specific amino acid residues that are essential for its function. Genetic approaches will be taken to examine the

similarities of the bO induced pathways to those normally induced by incompatible pathogens. When completed, these studies should provide new insights into the action of bO in plants and the mechanism of disease resistance induction in plants. They may also help us to determine the range of applicability of the bO gene to induce disease resistance in a variety of crop plants.

9902521 Characterization of a New Salicylic Acid-Binding Protein, SABP2

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Postdoctoral Fellowship; Grant 99-35303-8190; $70,000; 2 Years

Plants have an innate ability to defend themselves against disease-causing pathogens. However, pathogens are also programmed for survival. This battle between plants and their pathogens causes agricultural crop losses in excess of $100 billion annually. By genetically improving a plant's innate resistance mechanisms, agricultural crop protection can be improved. As for all technology-based advances, progress begins with a thorough research-driven understanding of the basic components of the system, here plant disease resistance.

Salicylic acid is a chemical component used by plants as an internal trigger for resistance against many plant pathogens. Three new plant proteins that interact with salicyclic acid have been identified, and while all three proteins will be studied, this proposal focuses on one, SABP2. SAPB2 will be obtained in pure form from tobacco, which will allow identification of the protein and also allow the gene that encodes SABP2 to be cloned. Tobacco is used as a model plant because it is easy to grow and each plant provides a large amount of leaf tissue for research purposes. With the SABP2 gene in hand, we will learn the conditions under which the plant uses this gene, as well as the degree to which this gene directly effects plant disease resistance. These later studies will also be pursued in the model plant *Arabidopsis*. Once a though understanding of SABP2 function is obtained, the knowledge will be transferred to agricultural crops for developing biotechnology-based crop improvement strategies.

9902398 Plant Pathogenicity Genes in *Streptomyces* spp.: Characterization and Horizontal Transfer

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Grant 99-35303-8084; $225,000; 3 Years

Streptomyces scabies and several other *Streptomyces* species infect potato tubers and root crops, causing significant economic loss. Though there are hundreds of species of *Streptomyces*, very few are able to cause plant disease. Two of these species are newly emerged pathogens. Our long term goal is to understand how these unusual plant pathogens parasitize plants and how plant pathogenicity has evolved in this genus. All plant pathogenic *Streptomyces* strains produce one or more members of a family of compounds that are toxic to plants; these phytotoxins appear to be intimately involved in the disease process. We believe that the phytotoxin biosynthetic genes, and possibly other pathogenicity related genes, are clustered in a "pathogenicity island" that comprises, or is contained within, a transferred DNA fragment. We have genetic evidence that supports horizontal gene transfer (transfer of DNA fragments between microorganisms) as a mechanism of evolution of pathogenicity. We will investigate the role of horizontal gene transfer in the production of phytotoxins and determine whether phytotoxin production is a pathogenicity factor among *Streptomyces* species. The specific objectives of this proposal are to locate and characterize the gene cluster involved in phytotoxin biosynthesis. We will also determine whether phytotoxin production is required for pathogenicity in *Streptomyces* species on several hosts. This research will lead to an understanding of the mechanism by which an important group of microorganisms cause plant disease and will facilitate the development of disease resistant crop plants.

9902586 Recombination in the Rice Blast Fungus

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Grant 99-35303-8053; $120,000; 2 Years

The rice blast fungus, *Magnaporthe grisea*, is notorious for its pathogenic variation and ability to overcome host plant resistance despite the lack of a sexual stage in most populations. This fungus has recently been hypothesized to recombine sexually in some populations in mountainous regions of south Asia. The ultimate objective of this project is to document the occurrence of sexual reproduction by detecting recombination among genes on different chromosomes of the fungus. To accomplish this goal, we will develop molecular genetic markers on at least four or five of the seven chromosomes in *M. grisea*. These markers will be found by a combination of searching for variation in conserved genes and by using nucleotide sequences from a genomics database. We will find polymorphisms in these markers using a technique sensitive enough to detect single nucleotide differences between strains. By assaying samples of *M. grisea* from natural populations with these markers we will be able to differentiate between clonal evolution and sexual recombination. In addition, by comparing rates of recombination between markers located on the same chromosomes with recombination rates

between chromosomes we hope to be able to distinguish between normal sexual recombination and parasexual recombination. This project will contribute to our understanding of how plant pathogens evolve to overcome disease management tactics such as host plant resistance.

9902564 Nuclear Import of Potato Spindle Tuber Viroid
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Grant 99-35303-4519; $140,000; 2 Years

Viroids are small RNA pathogens that infect many economically important crops. To establish a systemic infection of host plants, many viroids need to get into the host cell nuclei for replication. Although this nuclear import is key to viroid infection, little is known about the mechanisms involved. Our long term goal is to understand this fundamental process at the molecular level. We have developed an in vitro system to study viroid import in protoplasts. Our recent studies indicate that potato spindle tuber viroid (PSTVd) RNA is imported into the nucleus in a specific and receptor-mediated, and cytoskeleton-independent manner. These data suggest that PSTVd contains an import motif. In this project, we will characterize the mechanisms of nuclear import of a series of PSTVd mutants in order to identify the PSTVd sequence or structure that is critical for import. The data generated will be used in future protein binding experiments to isolate potential host factors involved in viroid import. The project is expected to generate valuable information on the mechanisms of nuclear import of viroids and advance our understanding of pathogen/host interactions in a broad sense. Furthermore, the information may provide a knowledge base for designing strategies to combat viroid or viral diseases.

9902404 Microscopic Examination of Oat Cell Death
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Grant 99-35303-8092; $128,711; 2 Years

Programmed cell death (PCD) is a genetically-regulated, controlled form of cell death distinct from the type of cell death typically referred to as necrosis. PCD entails the active participation of the cell in its own disassembly (and consequently, it's death) and its usual function is to eliminate unnecessary or aged cells. It is a normal form of cell death essential for the development and maintenance of multicellular organisms. PCD is distinct from necrosis where cell death usually results from irreparable damage. However, the inappropriate initiation of PCD can also lead to disease. Because of the importance of PCD to both normal health and disease, it has become one of the most studied topics in contemporary animal biology. Although not as well studied, PCD has also been implicated in a number of agriculturally important plant processes such as; development, senescence and disease. We have recently found that the toxin, victorin, produced by the fungal pathogen of oats called *Cochliobolus victoriae*, induces a response in oats that includes the biochemical hallmarks of PCD. Thus, understanding how this toxin works will likely provide insights into the regulation of PCD in plants and its involvement in plant disease development. The proposed work will use the tools of both fluorescence and electron microscopy to study this response at the cellular level and should contribute to our understanding of how PCD is regulated in plants and how victorin induces this response.

1999-02514 Inhibition of Appressorium Formation by Bioactive Peptides
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Grant 99-35303-8057; $145,000, 2 Years

Development of environmentally safe plant disease control strategies is an important goal as more intensive agricultural practices dominate food production worldwide. *Magnaporthe grisea, the* causal agent of rice blast disease, is a major threat to rice production. The costs of preventative chemical treatment for the disease and reduced yields when disease occurs makes blast an economically important, chronic, and global problem. M. *grisea* has become a major model system for understanding fundamental processes in fungal-plant interactions both because of its importance to agriculture and because it is amenable to genetic and molecular studies. A critical stage of the disease cycle is the production of the infection structure, the appressorium. Chemicals that block appressorium maturation are effective disease control agents. Screening of synthetic peptide combinatorial libraries led to the identification of a pool of compounds that inhibit appressorium formation. One goal is to identify the optimal peptides that inhibit appressorium formation. These peptides also provide pharmacological tools to examine the signaling pathway that regulates appressorium formation. Mutants affected in signaling appressorium formation will be tested for their response to peptides. Finally, by direct biochemical assessment of the effect of peptides on cA NIP levels we will test our hypothesis that peptides block accumulation of cAMP. The value of measuring cAMP levels is that it also tests the generally accepted view that cAMP levels increase to trigger appressorium formation. Although indirect evidence indicates that cAMP levels must increase during induction of appressorium formation in M. grisea, this has yet to be shown directly.

9902563 The Role of Satellite Panicum Mosaic Virus (SPMV) in a Virus Synergism on Grasses
Scholthof, K.B.G.
Texas A&M University; Department of Plant Pathology and Microbiology; College Station, TX 77843
Grant 99-35303-7974; $200,000; 3 Years

St. Augustine decline, caused by a complex interaction between panicum mosaic virus (PMV), a satellite virus of PMV (SPMV), and satellite RNAs (satRNAs) is a continual problem for homeowners, park managers, and urban landscapers. Recent data has shown that the determinants for severe mosaic, mottling, and stunting in millet species is due to the presence of the satellite virus, SPMV. More specifically, these symptoms appear to be linked with the expression of the SPMV coat protein. This proposal seeks to dissect the 824 nucleotide (nt) SPMV genome to further characterize the RNA and protein determinants for movement, encapsidation, and symptom development. A defective-RNA (D-RNA) derived from SPMV that is fully competent for replication and spread will be used to identify the relevant features on the SPMV genome. Objective 1 will characterize the naturally occurring SPMV D-RNA by reverse genetics. Objective 2 will define the role of a37 nt cis-acting RNA on the SPMV RNA that may have a role in host-dependent movement. Objective 3 will use a tomato bushy stunt virus (TBSV)-based virus vector system to further define regions required for SPMV encapsidation. In summary, this proposal will further our knowledge of both plant pathology and molecular virology, and may suggest future strategies for controlling plant virus diseases.

9902581 Biochemical and Biological Analysis of Host-Specific Plant Virus Transport
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Grant 99-35303-8022; $140,000; 2 Years

Despite the substantial damage that is caused every year by viral infections in crop plants, it is remarkable that most plants in nature do not support an infection by most viruses. Whether a plant is a host for a virus is often determined by their ability to establish a viable interaction for systemic transport of the virus throughout the plant. Although cell biology studies have started to yield insight into the pathway of plant virus movement, the biochemical interactions governing virus transport are unclear. A better understanding of the mechanisms underlying virus movement is fundamental to plant pathology.

The spread of tomato bushy stunt virus (TBSV) is mediated by a cell-to-cell movement protein and through host-specific contributions of its coat protein and a small non-structural protein. The first objective of this proposal aims to examine in vitro interactions established by these TBSV proteins that are involved in virus spread. The second objective focuses on the isolation and characterization of ribonucleoprotein complexes that occur in vivo. Combined, these analysis should yield insight into the host-specificity and biological relevance of biochemical interactions that may occur for virus proteins involved in movement.

9902518 The Role of Mutagenic DNA Repair in Accelerating Molecular Evolution in *Pseudomonas syringae*
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Grant 99-35303-8056; $185,000; 3 Years

The development and maintenance of populations of *Pseudomonas syringae* on leaves of plant hosts is a critical starting point prior to the initiation of pathogenesis. Leaf surface or epiphytic *P. syringae* cells also provide inoculum for dissemination and, in some cases, may serve as active ice nuclei capable of catalyzing frost injury to susceptible plant hosts. We have shown that tolerance to ultraviolet radiation (UVR) in *P. syringae*, conferred by the plasmid-encoded rulAB operon, is an important phenotype involved in the maintenance of epiphytic population size. RulAB has a world-wide distribution among *Psyringae* pathovars and confers UVR tolerance through a mutagenic DNA repair system; these systems repair otherwise irreparable DNA damage incited by UVR. A byproduct of mutagenic repair is an increase in the cellular mutation rate as a consequence of the introduction of mutations during the DNA repair process. Other researchers have noted that the possession of an elevated cellular mutation rate is a significant factor in the adaptive evolution of bacterial populations. The experiments outlined in this proposal are designed to genetically characterize the mutagenic DNA repair systems of *P. syringae*, and to determine if these systems can facilitate the adaptive evolution of strains in structured and unstructured habitats. Such knowledge of pathogen biology is necessary to improve the incorporation and risk assessment of disease management strategies, such as host resistance or biological control; strategies which may be defeated rapidly by an evolving pathogen.

9902411 Characterization of Cellular Receptors(s) for Tomato Spotted Wilt Virus in Thrips

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Grant 99-35303-8271; $316,000; 3 Years

Our experimental system involves tomato spotted wilt virus (TSWV, Family *Bunyaviridae,* Genus *Tospovirus)* and its insect vector, the western flower thrips (WFT). The overlapping host range of TSWV and the WFT includes some 900 species in 70 families, many of them important food, fiber and ornamental crops. Together TSWV and WFT are responsible for major economic losses throughout the world. Methods to control TSWV and thrips damage involve the wholesale application of wide spectrum insecticides. Such programs are damaging to the environment and largely ineffective because of the huge insect populations and the rapid development of resistance. Transmission of arthropod-borne viruses depends on specific molecular interactions between the virus and its vector. We are deciphering the molecular components of the interaction involved in the acquisition of TSWV by thrips. We have shown that glycoproteins on the surface of TSWV (ligands) interact with specific proteins (cellular receptors) on the thrips gut wall. We have cloned the genes encoding the ligand and the receptor proteins and will define the exact molecular determinants that specify their interaction. Our ultimate goal is to use the interaction between the viral ligand and the thrips receptor to create environmentally safe and effective control strategies. These will involve (1) blocking transmission of TSWV by constructing transgenic plants that express altered viral glycoprotein molecules that will interact with the receptor and prevent attachment of whole virus and (2) targeting toxins in a species-specific manner to the viral receptors on the thrips gut wall.

9902502 The Role(s) of Pathogenicity Genes in the Life Strategy of *Pseudomonas syringae*

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Grant 99-35303-8089; $155,000; 2 Years

Pseudomonas syringae pv. syringae (Pss) is well known for its economic impact on crop production due to its abilities to cause disease and frost injury. During the past decade, substantial progress has been made in the molecular characterization of genes reported to be required for pathogenicity in Pss. What is known about the effects of these genes in plant-bacterial interactions is based largely on laboratory assays in which the bacteria are infiltrated into leaves. Such assays, however, circumvent a significant portion of the natural interactions that occur between bacteria and plants in the field. This research continues our efforts to evaluate the field behavior of mutants of Pss that carry defects in specific pathogenicity-associated genes to better understand the function(s) of these genes in plant-bacterial interactions as they occur in the real world. We focus on genes in the hrp and *gacS/gacA* regulons. Some genes in both regulons affect the ability of the pathogen to grow in/on leaves *of* its susceptible host (i e., snap bean, *Phaseolus vulgaris L.)* in the field, an activity essential for disease epidemics to occur. A subset of genes in the *gacS/gacA* regulon appear to be directly required for lesion formation. The information obtained will lead to a better understanding of the ecology and epidemiology of a foliar bacterial pathogen and the disease it causes, which in turn, may provide new insights into ways of manipulating pathogen populations for disease control.

ENTOMOLOGY AND NEMATOLOGY

Panel Manager - Dr. Louis Bjostad, Colorado State University
Program Director - Dr. Mary Purcell

Research grants in this area support studies on plant stress arising from interactions with insects, mites, and nematodes. The goal of the program is to support research which will lead to ways of reducing losses in plant productivity from damage caused by these organisms, and to do so in such a way as to sustain agricultural systems.

Research on plant/insect and plant/nematode interactions is encouraged as is research into the basic biology of these particular invertebrates. Specific emphasis is placed on but not limited to the following areas: (1) mechanisms of plant defense and resistance against insect or nematode attack (biochemical or genetic), (2) insect or nematode growth, development and reproduction (physiological, biochemical or molecular studies), (3) physiological and ecological interactions of biological control agents with their hosts (includes beneficial insects, viruses, bacteria, fungi and other organisms), (4) chemical ecology and behavior, (5) population dynamics (includes evolutionary studies), and (6) fundamental basis of pesticide toxicity and resistance.

9902636 Genetics and Ecology of Pink Bollworm Resistance to Bt Cotton
Tabashnik, B.; Dennehy, T.; Carriere, Y.
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Grant 99-35302-8300; $200,000; 3 Years

Proteins from the bacterium *Bacillus thuringiensis* (Bt) offer safe, effective insect control that could be a cornerstone of sustainable U.S. agriculture. Crops genetically engineered to produce Bt toxins are grown on millions of acres, but evolution of resistance by pests threatens the continued success of this environmentally benign approach. So far, cotton that produces Bt toxin Cry1 Ac (Bt cotton) has been extremely effective in controlling the pink bollworm (PBW), *Pectinophora gossypiella*, a major pest of cotton in the southwestern U.S. In Arizona, Bt cotton accounts for more than 60% of the cotton grown statewide and the risk of PBW resistance is high. Laboratory selection has readily produced resistant strains of PBW. The EPA has mandated the refuge/high dose strategy to delay evolution of resistance to Bt cotton**,** but the information required to determine if this strategy is appropriate for PBW is not available. To help design sound strategies for managing resistance to Bt cotton in PBW we will: 1) Determine the mode of inheritance of PBW resistance to Bt toxin CrylAc; 2) Determine if fitness costs are associated with PBW resistance to CrylAc; 3) Determine if the same genetic locus controls resistance to CrylAc in different strains of PBW; 4) Develop and apply resistance management models using information from Objectives 1-3. This project will contribute knowledge vital to sustainable use of Bt crops, which can reduce use of conventional pesticides, thereby preserving environmental quality and protecting the health of farm workers and consumers.

9902718 Molecular Characterization of Aphid Resistance in Melon
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Grant 99-35302-8631; $150,000; 2 Years

Resistance to cotton-melon aphid (*Aphis gossypii*) in melon has a unique combination of features that make this an agriculturally important trait. The trait causes pronounced reduction in aphid populations and is linked with plant virus and powdery mildew resistances. Aphid resistance is encoded by a single dominant gene, *Agr* (*Aphis gossypii* resistance). The effects of resistance on aphid life history and feeding behavior have been studied extensively in two melon lines that differ primarily in the aphid resistance trait. We have taken the initial steps to construct a genetic map of *Agr* a chromosome in the melon genome. Molecular approaches will be used to construct a high resolution map of the DNA surrounding *Agr*, facilitating the gene's isolation and characterization. Two classes of DNA sequences, gene-specific and randomly generated (amplified fragment length polymorphism markers), will be used to identify chromosomal regions adjacent to *Agr*. Recent advances in understanding of plant resistance, including resistance to the potato aphid, raise the possibility that *Agr* a member of the largest known family of plant resistance genes. Conserved DNA sequences from this gene family will be used to determine if *Agr* a member of the family, which could suggest the mechanism of resistance. The isolation of *Agr* may require the construction of a specialized library (bacterial artificial chromosome library) composed of large DNA fragments from an aphid-resistant melon variety. Isolation and biochemical characterization of *Agr* will provide the basis for future work to determine the physiological mechanism of this resistance trait.

9902695 Dietary Contributions to Fitness of Males of Two Invasive Tephritid Species
Lauzon, C.; Opp, S.
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Strengthening Award; Grant 99-35302-8630; $100,000; 2 Years

True fruit flies, such as the Mediterranean fruit fly and the walnut husk fly, are invasive pest species capable of inflicting crop damage costing global economies millions of dollars each year. In California, the current preventative program against Mediterranean fruit fly costs over $13 million per year. A key aspect of this preventative program is the release of sterile male Mediterranean fruit flies to interrupt the reproductive cycle of the flies. Studies have shown that both health and survival of sterile males are generally poor, likely due to the stresses of mass-rearing and irradiation. Our goal is to improve the effectiveness of the sterile insect release technique by investigating the influence of adult diet on male fruit fly performance in terms of survivorship, flight, and physiological indicators of health. We plan to study two species of fruit fly, the walnut husk fly which has successfully invaded and colonized California, and the Mediterranean fruit fly which is a constant invasive threat in California. We will investigate the potential contribution of microorganisms, including bacteria and yeast, in fruit fly diets to: 1) fly physiological performance, 2) energy expenditures for flight, and 3) the development of healthy digestive tracts. Overall fly performance and health will be evaluated biochemically, microscopically, behaviorally, and ecologically.

9902469 Genetic Variation in Grape Phylloxera Life History Traits by Spontaneous Mutation
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Postdoctoral Fellowship; Grant 99-35302-8404; $90,000; 2 Years

The grape phylloxera is a serious root-feeding pest of cultivated grapes worldwide. Reproduction in California is primarily, or completely, asexual. This means that the progeny of a given mother are genetically identical, barring the variation introduced by new mutations. The use of rootstocks that resist the effects of the insect has been the most effective method of control of the insect. Resistance will fail if genetic variation in pest populations is sufficient to allow adaptation to resistant rootstocks. Resistance of rootstocks has failed in the past. The extent of genetic variation incorporated into asexual lineages of grape phylloxera by new mutations is unknown. The work proposed will provide estimates of the rate of production of genetic variation by new mutations for the traits that are important in the control of the insect, and the effect of new mutations on insect reproduction and development. Lines of phylloxera will be asexually propagated and allowed to generate genetic variation by accumulating mutations for up to 50 generations, controlling for the influence of other factors that might produce variation. Periodically lines will be assayed for reproduction and development. The variation among lines, and the change in mean over the course of the experiments will allow estimation of the extent of genetic variation, its rate of production, and the effects of new mutations on reproduction and development. Knowledge of the extent, rate, and consequences of the origin of genetic variation by mutation in asexual lineages will help to generate more powerful predictions about the capacity of this insect to adapt to resistant rootstocks.

9902541 Determination of the Roles of Secreted Proteins in Root-Knot Nematode Parasitism
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Grant 99-35302-8372; $150,000; 2 Years

Root-knot nematodes cause extensive damage each year to crop plants, therefore, controlling (these nematodes is an important U.S. agricultural goal. In order to prevent nematode attack on plants it is essential to identify and exploit weak-points in the nematode life cycle. Secreted proteins from nematode glands are potential targets for controlling nematodes because these secreted proteins are thought to be critical factors that allow nematodes to parasitize plants. In this proposal we have identified three classes of nematode genes that may be manipulated to kill the root-knot nematode. One class of genes encode nematode enzymes that are injected into plant cells that cause alterations in plant metabolism and aid in nematode parasitism. We propose to develop an inhibitor of such enzymes, to express this inhibitor in the plant, and to determine if this inhibitor can prevent nematode parasitism. The second class of nematode genes encodes enzymes that break down plant cell walls and aid the nematode in penetrating its host root. We will try to determine the biochemical activity of this putative nematode enzyme and determine if it is secreted into plant cell walls. The third class of genes encode secreted peptide hormones that may control aspects of nematode development. We propose to determine the location of these hormones in the nematode's body and to access the possibility of using these hormones as targets for future manipulation of nematode development.

9902703 Ecological Mechanisms Mediating Invasion of an Exotic Pest Insect

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Grant 99-35302-8188; $200,000; 3 Years

The United States faces an increasing influx of new organisms due to the expansion of trade and commerce. Among these new arrivals are pest species which destroy trees, crops, and structures, and spread disease. This project will examine factors contributing to the establishment of pests in a new habitat. In particular, the project will study the interactions between two pests of Eucalyptus trees, one of the dominant ornamental and shade trees in California. Eucalyptus remained free of insect pests until the 1980's, when the Eucalyptus borer *Phoracantha semipunctata* was accidentally introduced. This insect rapidly became a pest, killing large numbers of Eucalyptus trees, but during the 1990's, an effective management plan was developed, using natural enemies of the borer in combination with cultural practices, and replanting with less susceptible Eucalyptus species. However, in 1995, a second pest species, *Phoracantha recurva*, was also introduced. Within three years, this species had virtually replaced *P. semipunctata*, and damage to Eucalyptus trees was increasing Superficially, the two insect species are similar However, subtle differences in their biology, their susceptibility to the natural enemies being used for their control, or the susceptibilities of various Eucalypts must be responsible for the rapid increase of the second species Determination of the key factors enabling *P. recurva* to flourish at the expense of *P. semipunctata* will in the short term provide baseline data for modifying pest management plans, and in the long term, provide insight into how new pests become established.

9902666 Factors Limiting Invasions of Argentine Ants: Colony Size and Abiotic Factors

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Grant 99-35302-8675; $112,073; 2 Years

Biological invasions threaten the integrity of the world's biota. Given the magnitude of these problems, it is surprising that the proximate mechanisms responsible for the success of many invasive species are poorly understood, even for species that are commercially damaging The goal of the research described in this proposal is to isolate the factors limiting the ecological success of a widespread invasive social insect, the Argentine ant *(Linepithema humile).*

Like many competitively dominant species, Argentine ants appear to be limited more by abiotic factors, such as temperature and moisture availability, than by interspecific competition In this proposal, we employ a combination of laboratory experiments and field experiments to assess how physical conditions and colony size interact to determine the Argentine ant's competitive ability We exploit the ease with which Argentine ants can be reared in the laboratory to test how the effects of interspecific competition depend on abiotic factors and colony size To complement these highly controlled laboratory studies, we will conduct a series of manipulative field experiments to test further how the activity and competitive ability of Argentine ants depends on variation in colony size and ambient temperature.

The research proposed here integrates past and ongoing experimental studies and, taken together, will provide a unusually complete understanding of Argentine ant's ecological success This body of knowledge will provide essential information for the design of effective control strategies, promote an appreciation of the success of invasive ants in general, and clarify the important ecological forces structuring ant assemblages.

9902729 Genetics of Host Use in an Oligophagous Parasitoid, *Diaeretiella rapae*

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Grant 99-35302-8020; $125,000; 2 Years

Natural enemies of insects, including parasitoid wasps, commonly provide significant control of agricultural pests. However, species that attack a number of hosts can become specialized on one or a few hosts, either by genetic adaptation or by developing preferences based upon which hosts are commonly encountered. This local adaptation is likely in isolated populations and when differences in reproductive potential exist on different hosts. Host use by parasitoids is of major concern to practitioners of biological control. The ability to switch between hosts is important in annual crops, where hosts are not available during all seasons. Locally-adapted wasps may be less effective in controlling a number of pests simultaneously.

We propose to study the genetic basis of host use in *Diaeretiella rapae*, a parasitoid of a number of aphid species, but mainly the cabbage aphid (on cabbage) and the Russian wheat aphid (RWA) (on barley and wheat). Both aphids are significant pests and commonly occur in Colorado. In the last decade, new "strains" of the wasp were purposely introduced from Asia to control RWA. Background studies of *D. rapae* in Colorado indicate that populations are subdivided on a spatial scale as small as 1 km, and that these populations are adapted to either RWA or cabbage aphid. We propose to extend these studies to determine which traits (hunting behavior or survival) are responsible for differences in performance on the two aphid hosts. Furthermore, we will determine whether wasp populations on RWA and cabbage aphid exhibit genetic adaptation to these hosts.

9902658 Gene-transfer Vector and Marker Analysis for Insects of Economic Importance

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Grant 99-35302-8138; $200,000; 3 Years

The efficient creation of transgenic insect strains holds an enormous potential to develop beneficial insects with improved disease resistance, and the development of environmentally safe biological control programs for pest species. This project focuses on the development and testing of a gene transfer vector called *piggyBac*, that was discovered (as a transposable element) in the cabbage looper moth, *Trichoplusia ni* We have proven it to be an efficient vector in several tephritid pest species including the Mediterranean, Caribbean, and oriental fruit flies, as well as *Drosophila*. Routine use of this system will require a further knowledge of the presence and function of *piggyBac* in potential host species, optimization of the vector system, and an assessment of *piggyBac* stability To ensure stability of transgenic strains for mass-rearing and field release, *new piggyBac* vectors will be constructed that allow immobilization of the vector after chromosomal integration The development of *piggyBac as* a genetic tool for sophisticated genetic analyses and manipulation will be achieved by vectors that incorporate a yeast genetic expression system (GAL4/UAS) This will allow the identification and isolation of useful structural genes and regulatory DNA, as well as creation of strains with highly specific transgene expression Progress has also been made in the creation of a green fluorescent protein (GFP) marker system that has the potential for transgenic selection and genetic marking for field release in a wide range of species The GFP marker will be optimized for efficient detection, and its effect on the viability and reproductive fitness in host organisms will be assessed.

9902465 Systematics of Fire Ants in the *Solenopsis saevissima* Complex

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Grant 99-35302-8629; $200,000; 4 Years

The project will combine anatomical and molecular genetic data to resolve significant problems in the classification of fire ants, which originated in South America.. This work is important because many of the species in this complex of pest insects are poorly known and difficult to distinguish from one another; these are factors that have impeded their scientific study. The specific objectives are to: 1) develop new sources of characters for fire ant classification, 2) generate detailed descriptions and range maps of the species, 3) resolve nomenclatural problems to produce a stable system of species names, 4)produce a key for species identification suitable for use in the field and laboratory, and 5) infer the evolutionary relationships of the species This work will provide a strong foundation for a wealth of comparative biological studies of fire ants and their natural enemies.

9902667 Root-Knot Nematode Parasitism Genes: Molecular Characterization and Functional Analysis

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Grant 99-35302-8080; $200,000; 3 Years

Plant parasitic nematodes cause enormous losses in food and fiber crops in the U.S. and worldwide. The most cost-effective and sustainable strategy for limiting nematode damage to agricultural crops is the development of crops that resist nematode attack. Root-knot nematodes are the most economically important group of plant parasitic nematodes and will attack almost every food and fiber crop. These highly successful parasites have evolved a very specialized and complex feeding relationship with their host plants to cause the destructive root-knot disease. Root-knot nematodes initiate their parasitic relationship with the plant by injecting secretions into root cells These secretions stimulate the root cells to become specialized feeding cells that are the sole source of nutrients essential for root-knot nematode survival A deeper understanding of the basic mechanisms of nematode parasitism is critical for discovering new targets in root-knot nematodes to develop novel plant resistance using biotechnology. The overall goal of this research project is to use biotechnology to identify the parasitism genes that produce the nematode secretions responsible for the formation and maintenance of the feeding cells. The new knowledge that will result from this research project will significantly increase our basic understanding of nematode parasitism of agricultural crops and lead to new approaches for disrupting the feeding relationship of the nematode with its host to reduce crop damage. This knowledge will be used to apply genetic engineering to develop crop plants with new and durable types of resistance to manage the destructive root-knot diseases and increase agricultural productivity and sustainability.

9902724 Mutants and Signal Transduction: Two Keys to Understanding *Heterodera* Parasitism

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Grant 99-35302-7938; $200,000; 3 Years

Cyst nematodes (*Heterodera* spp.) are major pests of agricultural crops worldwide. Losses due to cyst nematodes reveal that more efficient management strategies are needed. Successful cyst nematode parasitism relies on unknown plant mechanisms. This project explores these plant mechanisms that allow cyst nematode parasitism to be successful. The long-term goal is to genetically engineer plants that are resistant to cyst nematodes. The availability of novel cyst nematode-resistant crop cultivars could effectively control cyst nematodes in agricultural fields. The sugarbeet cyst nematode (*H. schachtii*) is able to successfully parasitize the model plant *Arabidopsis thaliana*. Because *A. thaliana* is probably the best studied vascular plant known, the association of *H. schachtii* and *A. thaliana* is a powerful model pathosystem for cyst nematode pathogenesis. Findings made with this model system will be applicable in the U.S. to the soybean cyst nematode (the most damaging pathogen of soybeans) and the sugarbeet cyst nematode (a serious pest of sugarbeet production), among others. In this project, we are characterizing mutant *A. thaliana* plants that exhibit altered susceptibility to *H. schachtii*. Identifying mutated genes and characterizing their normal functions will reveal molecular components of the interaction between these two organisms. We plan to clone and characterize a previously identified mutant gene that is responsible for increased susceptibility to the nematode. Furthermore, we are exploring the functions of *A. thaliana* genes that change activity in response to the parasitizing nematode. Both approaches are anticipated to identify molecular targets and tools for the engineering of cyst nematode-resistant plants.

990728 Substrate Specificity and Inducibility of CYP6B8 from *Helicoverpa zea*

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Grant 99-35302-8627; $145,7145; 2 Years

The corn earworm, *Helicoverpa zea, is* one of the principal pests of corn in the UnitedStates; due to its polyphagous habits (*i.e.*, its ability to consume many host plant species), it is a pest on many other crops as well One physiological feature thought to contribute to its pest status is an efficient system for metabolizing toxins of natural and human origin In most caterpillars, the principal enzymes involved in this metabolism are the cytochrome P450s, a suite of enzymes localized in the insect's midgut. In this study, the substrate specificity of CYP6B8, an enzyme in *H. zea that* is inducible by xanthotoxin, a plant toxin, will be examined We will determine whether this enzyme can metabolize a broad range of natural and synthetic toxins, including pyrethroid insecticides, in order to establish whether it contributes to insecticide resistance as well as polyphagy We will also characterize the promoter region of the gene encoding this enzyme, to determine whether specific response elements exist that allow the insect to increase enzyme production in the face of specific toxicological challenges. Finally, we will attempt to estimate the diversity of P450 enzymes in this insect that contribute to polyphagy and to insecticide resistance Understanding some of the mechanisms that allow *H. zea* and other polyphagous pests to exploit a wide variety of crops and to develop resistance to a broad spectrum of insecticides could provide insights into developing new tools and approaches for environmentally sustainable management.

9902561 Understanding Host Race to Species Transitions in *Rhagoletis pomonella*

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Grant 99-35302-8514; $117,060; 3 Years

A major problem for agriculture is that some pest insects adapt to crops they did not previously infest These new populations, called "host races", differ genetically from original populations An example is the apple maggot fly, a major pest of apple. This native insect originally fed not on apples, but on hawthorn fruits. The apple and hawthorn races are genetically adapted to their plants in several ways. The goal of this research is to understand how host races can become more different than are the apple and hawthorn races, by focusing on yet another race of the apple maggot fly that feeds on dogwood fruits instead of hawthorn. Flowering dogwood is a native plant, and the host race on this plant must be older and better adapted to its host than the apple race. The specific goals are 1) to determine how much interbreeding occurs between hawthorn and dogwood races, 2) to determine whether interbreeding is due to flies mating on the"wrong" plant, 3) to determine whether there is any sterility between hawthorn and dogwood races, and 4) to determine how the flies are adapted to their food plants. This research will tell us whether host races on crops can be expected to continue to adapt and accumulate genetic differences. This basic scientific information will eventually be of great practical utility in breeding crops on which host races are unlikely to form.

9902641 Larval Exposure to Juvenile Hormone and Insect Growth Regulators and Division of Labor in Honey Bee (*Apis mellifera*) Colonies

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Postdoctoral Fellowship; Grant 99-35302-8187; $90,000; 2 Years

Pyriproxyfen is a synthetic mimic of the naturally occurring insect hormone, juvenile hormone (JH) and is used in many insecticides. Although pyriproxyfen does not kill adult honey bees, sublethal effects on adults and on developing bees (larvae and pupae) are not well known. Given the importance of pollination by honey bees to U.S. agriculture, it is important to assess potential effects of pesticides on the control of social behavior and development in honey bees. I have recently obtained data on fast and slow developing genetic groups of honey bees suggesting that faster developing groups (as measured by the onset of foraging behavior) have higher JH levels during two time points early in the larval period. These time points coincide with the onset of increasing JH which causes the totipotent female larva to differentiate into a queen. These results suggest that these time points may indicate a developmental window when exposure to JH or JH mimics like pyriproxyfen affect brain and behavior to influence how quickly a bee grows up to become a forager. I will test this hypothesis by treating larval bees with JH and pyriproxyfen and measuring their effects on behavioral development as measured by age at onset of foraging, sensitivity to different amounts of JH and pyriproxyfen; and effects of larval treatment with JH or pyriproxyfen on brain development and structure.

9902637 Regulation by 5-Hydroxytryptamine of Midgut Ion Transport in Larval *Aedes*

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New Investigator Award; Grant 99-35302-8371; $120,000; 2 Years

Very little is known about midgut ion transport mechanisms in larval mosquitoes. The PI has found that the anterior and posterior stomach regions of larval *Aedes* midgut possess distinct ion transport mechanisms that are regulated hormonally by 5-hydroxytryptamine (5-HT). The objectives of the proposed research are to identify the principal active transport processes of the anterior and posterior stomach, determine the ion transport processes stimulated by 5-HT in each region, and determine the physiological significance of coordination of midgut and Malpighian tubule function by 5-HT circulating in hemolymph. These goals will be met by determining, 1) the direction and magnitude of net flux of fluid and of each of the ions H+, Na+, K+, and Cl; 2) which ions are transported actively and which passively; 3) the effects of acute and chronic changes in ambient salinity and feeding status on fluid and ion ingestion rates, hemolymph 5-HT concentrations, hemolymph ionic concentrations and total body water in feeding and food-deprived larvae, and 4) the effects of inhibition of 5-HT re-uptake on each of these parameters. This basic research will greatly increase our currently very limited understanding of the regulation and function of the larval mosquito midgut, and thus contribute to the long-range development of control strategies for this important disease vector.

9902618 Mapping Diapause Genes in the Apple and Blueberry Maggot Flies

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Grant 99-35302-8079; $200,000; 3 Years

The apple maggot, *Rhagoletis pomonella,* is a major pest of apples in the United States, while the blueberry maggot, *R. mendax,* causes significant damage to blueberries. These two flies differ in several traits adapting them to attacking apple vs. blueberry fruits. In particular, *R. mendax* has a deeper diapause than *R. pomonella.* Because blueberries bear fruit earlier in the season than apples, the deeper diapause of *R. mendax* allows blueberry-fly pupae to withstand longer periods of warm weather before winter than apple-fly pupae. This study will map the location of genes responsible for the diapause difference between *R.. pomonella* and *R.. mendax.* This will involve crossing blueberry and apple flies to produce hybrid offspring, and then inter-mating the hybrids. The resulting F_2 progeny will be scored for diapause traits and DNA markers to identify regions of the *Rhagoletis* genome containing genes affecting diapause.

Mapping diapause traits represents an important step toward the long-term goal of cloning and characterizing the actual genes affecting diapause in *Rhagoletis.* Elucidating the genetic and physiological bases for diapause will have important consequences for designing effective management schemes not only for *Rhagoletis,* but for related tephritid pests as well (e.g., the Mediterranean fruit fly). First, it will allow eclosion times for flies to be accurately predicted in the field, resulting in more judicious application of pesticides. Second, understanding the, genetics of diapause will allow novel control mechanisms to be designed predicated on disrupting the match between fly development and the fruiting phenology of host trees.

9902683 *Wolbachia* Infections in Mosquitoes as an Applied Tool for Modifying Natural Populations

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New Investigator Award; Grant 99-35302-7988; $125,000; 2 Years

Mosquitoes are important pests of livestock, causing significant economic impact from biting and blood feeding that can result in annoyance, immune and toxic reactions in the host, and the transmission of disease. The economic repercussions of these attacks include weight gain reductions, lower milk and egg production, reduced fertility, and death. At present, the control of mosquitoes relies primarily on chemical and biological insecticides. However, the development of insecticidal resistance in mosquitoes combined with an increased difficulty in developing and releasing new larvicide and adulticide compounds have generated a growing interest in alternative strategies to complement current control programs. Intracellular bacteria of the genus *Wolbachia* have been suggested as a tool for modifying populations of mosquitoes and other economically important insect pests. Proposed strategies include the use of *Wolbachia* for both population reduction and population replacement. Both of these strategies are based on the ability of *Wolbachia* to induce cytoplasmic incompatibility, whereby the *Wolbachia* infection renders male mosquitoes impotent. The primary goals of this project will include evaluating the potential of *Wolbachia* to modify populations of the recently imported *Aedes albopictus* (Asian tiger mosquito) and developing techniques permitting the application of these strategies. These studies will also provide useful information for the design of similar strategies in additional insect pests of both livestock and plants.

9902684 Function of Segmentation and Segment Nesting in Bracovirus Genomes

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Grant 99-35302-8299; $200,000; 3 Years

A high percentage of lepidopteran pest larvae are killed early in the growing season by braconid parasitoid wasps, greatly reducing economic losses and the need for insecticides. Increasing the efficacy, virulence, and host range of parasitoid wasps are therefore an important goal of applied and basic biocontrol research. The wasp egg hatches inside the parasitized lepidopteran larvae, resulting in stunted growth, reduced feeding, altered hormones and behavior, immunodeficiency, and finally, death of the host larva. Female wasps co-inject into the host, along with the egg, a remarkable symbiotic virus, the *Microplitis demolitor* bracovirus (MdBV), which is the infective agent directly responsible for stunting and suppressing the immune response of the host. The genome of MdBV is like no other in that it is broken into 14 or more segments, most with a separate permanent location in the wasp genome; others are nested within a larger parent segment, and are subsequently released, or spliced out. Thus far we have managed to locate two of the viral genes which suppress the immune system of soybean loopers. To speed up the search, we propose to sequence the entire genome of this virus--thus acquiring in a single effort the entire suite of viral genes which stunt growth and suppress the immune system of loopers, bollworms, earworms and other lepidopteran pests. Next, with all the newly identified MdBV genes in hand, we propose to determine the effect of each gene on soybean looper growth, feeding, and immunodeficiency.

9902471 Evolution and Spread of Insecticide Resistance in the Colorado Potato Beetle

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Grant 99-35302-7907; $125,000; 2 Years

Colorado Potato Beetle has evolved resistance to all pesticides thus far developed. The current pesticide, Admire®, is failing locally and will soon be lost as a control tactic. A newly introduced transgenic potato plant is highly effective at suppressing these beetles, and is the last available control measure. We need strategies to slow the spread of resistance or the potato crop may be lost to the beetles within a few years. The newest tactic for managing resistance is to plant small, untreated, "refuge" plots within fields. Pests in these refuges soon greatly outnumber those in the treated crop. Newly-arisen, resistant individuals in the field will tend to mate with non-resistant individuals from the refuges, diluting resistance genes and slowing their spread. The success of this tactic depends critically on refuge size and placement, because dilution fails when resistant and susceptible individuals do not mix freely. We therefore need information about how resistance spreads to determine rules for the optimal size and placement of refuges. Our objective is to determine rules for the optimal size and placement of refuges. In one approach, we apply Admire® selectively to create steep environmental gradients, and use new population genetics methods to measure the rate that resistance spreads across the steps. In another approach, we use miniaturized radio-tagging technology to follow the beetles. We incorporate this information into computer models to determine the optimal size and placement of refuges. Our recommendations will apply regardless of the pesticide used against Colorado Potato Beetles, including transgenic plants.

9902709 Polydnavirus-based Transformation of Insect Cells

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New Investigator Award; Grant 99-35302-8522; $120,000; 2 Years

Polydnaviruses, unusual DNA insect viruses found in some parasitic wasps, are considered non-traditional viruses because they do not replicate outside of specific cells within the female reproductive tract. It was recently observed that polydnavirus DNA from the parasitic wasp *Glyptapanteles indiensis* could integrate itself into chromosomal DNA of cells derived from the natural host, gypsy moth, and was stably maintained. The phenomenon of integration makes this polydnavirus practical for development as a virus-based tool for transformation of insect cells. The goal for this work is to develop a new system for transformation of insect cells through (1) characterization of the *G. indiensis* polydnavirus (GIPDV) and the genes coded within the integrating DNA of its genome; and (2) analysis of *G. indiensis* polydnavirus integration by characterization of integration sites and the viral segments that integrate in gypsy moth cells under laboratory conditions. This will form the necessary groundwork for future development of a GiPDV-based vector for transformation of and gene expression in insect cells. The proposed research has importance to agriculture as a potential new and alternative insect cell transformation system, as part of long-range reduction in use of chemical pesticides by biological suppression and control of insect pests, and as groundwork for long-term transgenic insect strategies for biologically-based pest control efforts.

9902547 Microarray Analysis of Soybean Gene Expression Induced by Soybean Cyst Nematodes

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Grant 99-35302-8189; $200,000; 3 Years

The soybean cyst nematode (SCN) is the major pest of soybean in the US and worldwide The economic loss to U.S. farmers is approximately $1.5 billion per year. Current control practices are not adequate. Our long term goal is to understand the signals and mechanisms involved in the resistance response of soybean to SCN and to devise methods for broadening resistance of soybean to SCN through molecular techniques. The two goals of this proposal are (1) to produce an array of identified and unknown cDNAs, many which are expressed more highly in resistant cultivars in response to SCN invasion; and (2) to identify and characterize these genes in two resistant genotypes and a susceptible genotype with documented differences in their response to SCN race 3 at the cellular level. Variations in patterns of gene expression will be correlated with mechanisms of defense and function of known genes. Portions of genes (cDNAs) will be printed as a grid on a glass slide. Each cDNA will have a unique position on the slide and will be correlated with an archived clone for retrieval later. Gene expression levels will be measured by hybridizing fluorescently labeled probe representing mRNA (the product of expressed genes) to the cDNAs printed on the glass slide . The mRNA will be from root tissue of different soybean genotypes challenged and not challenged with SCN. This information will be useful to scientists breeding or engineering soybean for broader resistance to SCN.

9902687 Effects of Elevated Atmospheric CO_2 on Grasses and their Insect Herbivores

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Grant 99-35302-8050; $200,000; 3 Years

The economic importance of grasses for human nutrition (grains), livestock (forage), ground cover, and as weeds is unsurpassed by any other group of plants. Insects are the primary herbivores in North American grasslands. The concentration of CO_2 in the earth's atmosphere is expected to double in the next century, causing changes in the nutritional quality of grasses and changes in the impact of grass-feeding insects on grasslands. The proposed research seeks to understand (1) how elevated atmospheric CO_2 levels will change the nutritional quality of grasses and (2) how changes in grass nutritional quality will affect the growth and reproduction of grass leaf-feeding insects. In addressing these goals, the proposed research will make the first extensive comparison of changes in nutrients (e.g., protein and carbohydrates) and fiber in a wide variety of economically important grasses grown at current and elevated CO_2 levels. One important aim of the research is to better generalize about which types of insects will be affected most (positively or negatively) by future CO_2 levels. The research will make the first detailed examination of the mechanisms that explain why certain types of insects perform better or worse on different types of grasses and at different CO_2 levels. Finally, the proposed research will test the prediction that generalist insects will be strongly affected by elevated CO_2 levels, possibly resulting in increased feeding pressures on grasses or alternate (broad-leafed) host plants.

9902622 Frequency of Alleles for Resistance to *Bacillus thuringiensis* Toxin in European Corn Borer
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Grant 99-35302-7937; $150,000; 2 Years

Evolution of resistance by insect pests to insecticides, plant resistance and other insect control tactics costs U.S. agriculture over $100 million annually. Presently, management of this problem has relied on the use of static, unchanging management strategies, which have been sporadically successful. It has not been possible to adapt and improve management to changes in resistance as it has occurred, because no effective monitoring method exists. The proposed research will start to fill this gap by developing and comparing three independent monitoring methods to estimate the frequency of resistance to transgenic Bt corn. The projected precision associated with our proposed work is more than an order of magnitude more sensitive than any previously reported method.

9902652 Effect of Novel Fungal Endophytes on Pest and Beneficial Insects
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Strengthening Award; Grant 99-35302-8164; $100,000; 2 Years

Fungal endophytes of forage grasses have detrimental effects on livestock. Ergot alkaloids produced by the fungi appear to be the primary cause for these effects. In addition, fungus-produced loline and peramine alkaloids provide infected plants with enhanced resistance to insect herbivores. The fungi, and the toxins they produce, result in annual revenue losses of over $600 million in the USA current strategy by plant breeders is to develop grasses with fungal strains that reduce the negative effects on livestock but retain resistance to insects. The proposed research utilizes newly developed experimental endophyte strains that were artificially introduced (i.e., novel) into two tall fescue cultivars and tests their effects on pest and beneficial insects. I will test for resistance to caterpillars and aphids in two separate laboratory experiments in which prior plant damage and the presence and genetic strain of fungi will be manipulated. Biomass gain, consumption and survival will be monitored in caterpillars, while reproduction of aphids will be assessed. Nutritional chemicals (protein nitrogen) and potential allelochemicals (ergots, lolines and peramine) in plants will also be assessed. In an additional experiment, potential indirect effects of endophytes on parasites of aphids will be assessed. Survival and growth of parasites will be assessed under host diets that vary with respect to plant and fungal genotype and the presence of fungal endophyte. Results will 1)assess effects of experimental combinations of plant and fungal genotypes on pest and beneficial insects, 2) shed light on mechanisms by which endophytes influence plant-insect interactions and 3) assist efforts to manage endophytes in pasture systems.

9902470 Leafties as Ecosystem Engineers
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Grant 99-35302-8017; $125,000; 2 Years

On Missouri oaks (*Quercus* spp.), one species of leaf-tying caterpillar, *Pseudotelphusa* sp., physically engineers its environment by constructing leafties (two or more leaves sandwiched together with silk). Subsequently, these ties are preferentially colonized by a diverse insect assemblage of other leaf-tying species, non-leaf-tying herbivores, predators, and scavengers. We will conduct three experiments to quantify the impact of the initial leaftying insect species on the associated arthropod community structure and damage to the host plant, white oak. We will test the overall hypothesis that the presence of this one species of leaftying insect has multiple trophic level, competitive, and mutualistic effects through its construction of leafties. We predict that removal of this single species of caterpillar will affect tree growth because leaf damage has been shown to reduce growth and survivorship in white oak. Such an impact is due in part to its own feeding activity, but more importantly to its engineering impact on associated leaf-eating insects. By investigating the role of this caterpillar species as an ecosystem engineer, our experiments will clarify a little-studied, but perhaps major functional pathway by which organisms interact with each other. Because control of this single species of insect could have a significant influence on oak wood production (by negating its impact as an ecosystem engineer) and because leaftiers are a common component of the herbivore faunas of most broad-leaved deciduous trees, the results of this research have implications for forestry practices throughout the eastern North America.

9902559 Light Modulation of Herbivore-Elicited Chlorosis
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Grant 99-35302-8628; $150,000; 2 Years

Previous research has demonstrated at the plant level that leaf chlorosis is a light-activated process. Because the chloroplast is the essential organelle for chlorophyll synthesis, degradation, and photosynthesis, isolated wheat chloroplasts will be used to examine light variation on the chlorophyll synthesis and degradation caused by aphid salivary components.

The *in organello* system consists of: isolated chloroplasts of 'Betta(susceptible) wheat, and its three resistant isolines with *Dn1*-antibiosis, *Dn2*-tolerance, and *Dn5*-antixenosis genes; two sap-feeding aphids, a plant symptom-eliciting aphid, the Russian wheat aphid, and a plant non-symptom-eliciting aphid, the bird cherry-oat aphid; and light varying of intensity. The impact of aphid salivary components on chlorophyll degradation pathways and derivatives in wheat chloroplasts will be evaluated under varying light intensity. Aphid salivary components that cause chlorophyll degradation will also be identified. The information from this research will contribute to understanding plant transduction and responses to aphid salivary elicitors and other biotic stress signals. The use of chloroplasts from wheat isolines will provide vital and unique information about the mechanism of wheat plant resistance to aphids. The system used in this research can easily be adapted to examine other herbivore (e.g., greenbug, potato leafhopper, and *Lygus* spp.) elicited damage to plants.

9902662 Regulatory Networks in Nematode Feeding Sites

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Grant 99-35302-8233; $200,000; 3 Years

The goal of this research is to reveal host functions necessary for root-knot and other nematodes to cause stressful parasitic interactions with plants, and to use this information to construct genetic cassettes able to restrict these parasites in crop plants. Plant-parasitic nematodes reduce annual US agricultural production by more than $5 billion. The most important group are the root-knot nematodes which infect in excess of 2,000 plant species. Following host invasion, larvae induce highly modified giant cells to establish permanent feeding sites. Root cells from which the nematode is feeding expand greatly and undergo nuclear division without cell division. Giant cells serve as the obligate nutritive source for the developing nematode. We previously identified and characterized 220 genes with up-regulated expression in tomato giant cells. Our extensive analysis of these genes has generated a global, molecular view of the nematode feeding site. In this new project we will focus our attention on three genes in particular which appear to play regulatory roles in controlling central aspects of giant cell biology, including cell cycle regulation, control of differentiation, and the natural resistance response. Using a gene-ablation approach in transgenic plants, we have established that these genes encode essential giant cell functions, and thus are targets to make nematode-resistant, crop plants. In this renewal, we will define the regulatory cascades controlled by these genes. We will define protein-protein interactions, and identify promoters and genes in the function networks. Understanding the lynch-pins of the host response will reveal novel nematode-management strategies.

9902727 Mechanisms and Dynamics of Group Foraging in the Pine Sawfly *Neodiprion lecontei*

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New Investigator Award; Grant 99-35204-8229; $129,810; 2 Years

The red-headed pine sawfly is one of the most destructive defoliators of hard pines in eastern North America, including several ornamental and pulpwood pine species. Unlike many defoliating pest insects, this species feeds in close-knit groups (colonies), and grouping is of great importance to its pest status. Defoliation rate and destructiveness increases disproportionately with group size as a result of greater feeding efficiency and decreased predation. It is therefore of great interest to understand how these insects group and stay together as they feed, and to what extent groups merge to form super colonies. Preliminary studies suggest colonies stay together through the use of chemical markers, as is true of some moth defoliators. The goals of this project are to (1) determine through behavioral experiments exactly how these sawflies communicate to stay grouped as they move among feeding sites, specifically looking at the role of chemical trails; (2) track colony movements to document foraging periodicity and determine the extent of group splitting and refusion; and (3) determine with genetic markers the extent to which super colonies form under natural conditions. Since defoliation potential is dependent on group size in this species, a better understanding of mechanisms underlying group formation will improve management and control efforts based on the behavior of the insect. Learning how these insects group, for example, is the first step toward figuring out a way to disrupt colonies, perhaps by interfering with their chemical trails.

9902720 Inactivation of Insect Diuretic Hormones

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Grant 99-35302-8154; $160,000; 2 Years

In this project we will study how the tobacco hornworm breaks down its two diuretic hormones (Mas-DH and Mas-DPII). These hormones are small proteins containing 41 and 30 amino acids, and are important in controlling the rate of urine production. We earlier studied the metabolism of Mas- DH by its target tissue, the Malpighian tubules. We will conduct similar studies on Mas-DPII, which has a remarkably different amino acid sequence from Mas-DH. Mas-DPII may be degraded by a different enzyme as it lacks the structural element which is degraded in Mas-DH. We propose to extend our earlier studies on Mas-DH, which were performed at a low concentration, but still about a thousand times higher than the likely hormone concentration in the insect. To study this process at lower levels we shall have to make radioactively

labeled hormone. The breakdown of radioactive hormone will be monitored by reversed-phase liquid chromatography, testing to see which fractions coming out of the chromatography column are radioactive. There are two enzymes which degrade Mas-DH as judged by our prior studies; we propose experiments to purify these enzymes and determine how quickly they can break down the hormone at low levels. If these enzymes can break down the diuretic hormone at very low levels, then we may be able to design chemicals that block this process and kill the insects. This process should be highly specific for insects as mammals produce urine by a completely different mechanism.

9902672 Regulation of Baculovirus Late Gene Transcription

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Grant 99-35302-7952; $200,000; 3 Years

Baculoviruses are large double stranded DNA viruses that are highly pathogenic to many insect pests in the Order Lepidoptera. Baculoviruses represent one of the most promising natural enemies that are being actively examined and engineered for biological control of insect pests in agriculture. Our studies will address a number of fundamental questions that limit our understanding of the baculovirus infection cycle, and will enhance our ability to rationally engineer and manipulate baculoviruses for insect control and biotechnological applications. Central to understanding the infection cycle of these viruses is a clear understanding of how viral gene expression is regulated in the infected insect cell. We will conduct a detailed analysis of the regulation of late gene transcription. Using a detailed series of promoter truncations and point mutations, followed by analysis of the resulting promoter function, we will precisely characterize late promoter elements that mediate, a) late promoter recognition, and b) activated late transcription. We will also use a genetic approach to identify and characterize late transcription factors that regulate levels of late transcription. We will use stably transfected cell lines to generate null viruses containing knockout mutations in several essential late expression factor (*lef*) genes. These viruses will be used to more precisely examine the roles of specific *Lef* proteins in regulating late gene expression.

9902648 Engineering Midgut Chitin-Binding Peptides for Insect Control

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Grant 99-35302-8083; $200,000; 3 Years

Insect chitin has been identified as a potential target for insect management. Since chitin is a major component of structures such as the cuticle and the peritrophic membrane (PM), any interference with its formation or degradation would be detrimental to phytophagous insects. The insect PM has many functions and has long been considered an important microbial barrier to midgut infection and as a selective barrier of dietary toxins. We have found that Calcofluor (a chitin-binding agent) can disrupt or completely inhibit the formation of the PM in several important insect pests. Therefore, our studies suggest that targeting chitin-protein structures with the PM may constitute an important new strategy for control of phytophagous insects. The goals of the proposed research are to identify and functionally characterize the biochemical and molecular properties of chitin binding sequences (peptides) present in PM proteins from the cabbage looper, *Trichoplusia ni*. Selected peptides will be produced, purified and tested for chitin-binding activity. Highly active peptides will be tested for biological activity in insect larval bioassays to determine their effect on insect physiology and growth. Bioassays will also determine if these chitin-binding peptides can enhance the infection of insects by microbial agents. These studies will determine if these chitin binding peptides or antibodies against these proteins can interfere with PM formation in phytophagous insects to suppress their growth and development. If our anticipated findings are successful, this new technology will provide novel genetic tools for insect control by their use in recombinant microorganisms and transgenic plants.

9902685 Influence of Leaf Domatia in Mediating Interactions Among Beneficial Mites and Grape Pests

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Cornell University; New York State Agricultural Experiment Station; Department of Entomology; Geneva, NY 14456-0462
Grant 99-35302-8051; $125,000, 2 Years

Many plant species possess leaf domatia, tufts of non-glandular hairs or pits often situated in vein axes on the underside of leaves. It is hypothesized that domatia benefit plants by providing a refuge for fungal-feeding and predatory mites which, in turn, provide protection against plant parasites. Although present in a number of native grape species, domatia are lacking in most cultivated grapes. In previous research we determined that: 1) domatia in a species of wild grape promoted higher densities of fungal-feeding and predatory mites; and 2) the increased densities of fungal-feeding mites resulted in significant reductions in grape powdery mildew, the major disease of cultivated grape. Although these results demonstrate that domatia benefit natural enemy mites, the reasons for this benefit remain unclear. In this renewal grant we test two hypothesized mechanisms: That domatia protect these taxa from stressful humidity conditions; And that domatia protect these taxa from predation and competition with other arthropod natural enemies. Both phytophagous mites

and pathogens are serious pests of grapes in the U.S and pesticides are the primary technique used to control them. In the long-term, however, we believe that domatia can be used in commercial grapes to enhance biological control of both fungal pathogens and spider mites thereby reducing reliance on pesticides. To achieve this goal we need a more complete understanding of how domatia influence beneficial mites and their interactions with other organisms. Although this proposal focuses on grapes, the results should be applicable to other crops.

9902697 Resistance Management for Bt Plants: Testing the Next Generation of Management Options

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Grant 99-35302-7999; $150,000; 2 Years

U.S. agriculture must rely on the development of new technology to help increase the yield and quality of food and fiber products by reducing the stress on plants due to insects, diseases and weeds. Such technologies must be compatible with environmental and sustainable development as well as be profitable to growers. The advent of modern biotechnology has led to the production of plants that express Bt toxins to control chewing insect pests of some of our most widely grown crops in the U.S. (e.g., cotton, corn and potatoes). At least 16 companies are presently involved in developing transgenic crops with Bt genes. The commercial and environmental interests in this technology are profound. In 1998 nearly 9 million acres of transgenic plants were grown in the U.S. Despite considerable advantages of Bt-transgenic crops, both to the environment and to farm worker safety, concern is widespread that these gains will be short-lived due to evolution of resistance in the pests. Our work with the diamondback moth (DBM) on Bt-transgenic broccoli has provided the key example of an insect evolving resistance to transgenic crops. Our studies have examined the effects of refuge size, placement, and resistance gene frequency on the development of resistance and helped influence policies for resistance management programs for Bt plants. This proposal will contribute to the development and implementation of the next generation of Bt plants which will contain pyramided Bt genes and other non-Bt genes which can be used to delay the onset of resistance.

9902650 Pheromone Perception: Structure of Pheromone Binding Protein

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New Investigator Award; Grant 99-35302-8106; $200,000; 3 Years

An understanding of the molecular details of pheromone perception is essential if disruption of mating via sensory inhibition is to be achieved. Chemical interference with the molecular events which occur during pheromone perception in male antenna offers a unique strategy for the disruption of the pre-copulatory behavior of pest insects, and constitutes an environmentally benign alternative to increased use of traditional pesticides. To understand how to produce such "odor blindness", the three dimensional (3D) structures and functions of the proteins involved in the delivery and presentation of these volatile signals to dendritic receptors must be known. Specifically, to unravel the molecular details by which pheromone binding proteins (PBPs) recognize specific pheromone components and display them in such a way as to activate the receptor mediated G protein coupled phospholipase C which leads to ion channel opening by second messenger inositol triphosphate. Complete understanding of protein function and mechanism of action can only be accomplished with a knowledge of its three dimensional structure at atomic resolution.

The specific approach of this proposal is to use high resolution multi-dimensional NMR methods together with distance geometry techniques to determine the 3D solution structure of the pheromone binding protein. This will be accomplished using the best -studied PBP system as a model i.e., the *Anthereae polyphemus* PBP (Apo-PBP).

9902129 Salivary Gland Gene Expression During Tick Feeding

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Grant 99-35204-8567; $174,894; 3 Years

Ticks cause considerable losses in the livestock industry, both directly through blood loss and indirectly through transmission of pathogens such as *Anaplasma marginale*. The salivary gland is a key organ to both of these processes, as it is how the tick gets rid of excess water from the blood-meal, and is the primary route of transmission of pathogens. Ticks have a number of strategies and adaptations to allow long-term feeding on the host, and many of these may also aid in pathogen transmission, but little is known of the details of most of them. We will sequence randomly selected genes expressed in tick salivary glands at various stages of the feeding cycle, with an aim of getting 10,000 independent sequences (3-5000 different expressed genes). These will be identified, as much as possible, by comparison to known sequences from all organisms. This collection of data should help us identify many of the key factors involved in feeding success and pathogen transmission. We will generate an array of the genes we identify and determine their expression at different feeding stages, to get a better idea of their role in feeding.

9902716 Gordon Conference on Metamorphosis

Riddiford, L.M.
Gordon Research Center; University of Rhode Island; West Kingston, RI 02892-0934
Grant 99-35302-8250; $5,448; 1 Year

This grant provides partial support for the first Gordon Conference on Metamorphosis July 10-15, 1999 at Connecticut College, New London, CT. The Conference brings together people working on metamorphosis in insects and amphibians and a variety of other organisms to discuss the common cellular and molecular events that occur in metamorphosis and the questions that it poses about coordination and control systems in development. The 8 scientific sessions deal with hormonal regulation of metamorphosis at both cellular and organismal levels, mechanisms of gene activation and repression, intracellular receptors and gene regulation, transcription factor cascades in metamorphosis, the evolution of metamorphosis, cellular specification and differentiation during insect metamorphosis, cellular alterations during amphibian metamorphosis, and cell death during metamorphosis. There are 27 invited speakers and 21 contributed posters with 6 of the latter presenters (4 of whom are graduate students or postdoctoral researchers) invited to give short talks on their provocative results. The cross-fertilization among the workers on the various metamorphic systems should foster new insights into these complex systems and the similarities and differences between vertebrate and insects at the molecular level. These insights can lead to an elucidation of the critical steps that may be utilized as possible intervention points for control of insect and nematode pests of U. S. agriculture.

9902693 Cytochrome P450 Gene Regulation in Drosophila

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Grant 99-35302-8081; $200,000; 3 Years

Billions of dollars are spent every year to control insects with various types of insecticides. This strategy, however, eventually becomes almost ineffective because the target insects develop resistance to a variety of insecticides. In most cases, resistance is mediated by a class of enzymes called cytochrome P450s (CYPs) which are products of CYP genes. CYPs are found in all living organisms and they are capable of metabolizing a variety of chemicals including insecticides. It has been shown that the levels of one or more CYPs, and activity of the respective CYP genes are higher in resistant strains than in their susceptible counterparts. However, how insects up-regulate their *CYP* genes and become resistant to a variety of insecticides are not known. To better understand this mechanism, the proposed research will use fruit fly (*Drosophila melanogaster*) as a model insect, and *Cyp6a2* and *Cyp6a8* as the prototype CYP genes, which are up-regulated in resistant strains. Specifically, molecular genetic techniques will be used to map the chromosomal location of the regulatory gene(s) that control the activity of *Cyp6a2* and *Cyp6a8* genes. This will allow identification and cloning of the regulatory gene and help understand the mechanism that insects use to up-regulate their CYP genes. Information gathered in this research may help develop genetic engineering strategies to interfere with the up-regulation of resistance-associated CYPs and prevent the development of insecticide resistance in fruit flies, and agriculturally relevant insects, such as houseflies and mosquitoes.

9902691 Predator Sharing Among Insect Pests: Increase or Decrease in Predation Pressure?

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Grant 99-35302-8104; $125,000; 3 Years

Sharing of generalist predators by insect pests is frequently invoked as a key process affecting biological control of the pests. Such sharing, however, has not been well-studied and hence is of uncertain importance in most agricultural systems. Predator sharing may enhance biological control of a given pest if the predator occurs in high numbers in response to the availability of alternate prey even when the pest itself is at low numbers. On the other hand, predator sharing may lessen the impact on a given pest if the predator spreads its attacks among several prey species. The proposed research will examine whether and how the two principal insect pests of hay alfalfa in the inter-mountain west, alfalfa weevils and pea aphids, affect each others' numbers by sharing a major group of generalist predators, lady beetles. The central question is whether the co-occurrence of these pest insects in alfalfa fields weakens or intensifies the rate of predation of each prey by the generalist predators. Field and laboratory experiments will address this question by probing behavioral and population responses of the predators to each prey species in the presence versus the absence of the alternative prey. Experimental results will contribute to a broad assessment of the importance of predator sharing for biological control of insect agricultural pests in general. For alfalfa in particular, this may lead to practical means of enhancing management of the major insect pests based on their interactions with shared predators.

9902550 Improving Tolerance to UV-B of an Insect-biocontrol Fungus, *Metarhizium anisopliae*

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Grant 99-35302-8052; $150,000, 2 Years

Entomopathogenic microorganisms, including fungi, are alternatives to chemical pesticides in sustainable and integrated pest management programs worldwide. Solar radiation, however, severely reduces effective inoculum dose in the field through spore mortality; and as the ozone layer in the upper atmosphere decreases, negative effects of UV-B irradiation will increase The overall objective of our proposed research will be to gain an understanding of means to ameliorate solar (primarily UV-B) irradiation-caused damage to an important insect-biocontrol fungus, *Metarhizium anisopliae (Ma).* We will: 1) Determine the action of UV-B radiation on survivability of spores. Variability will be studied in natural isolates and mutants from differing geographic areas to detect isolates with strong and weak resistance to be used in the biochemical analysis; 2) Investigate biochemically how the fungus responds to oxidative stress imposed by UV-B irradiation. The focus will be on potentially protective enzymes, e.g. catalase and superoxide dismutase, as well as enzymes affecting glutathione and ascorbate levels. These two approaches should identify genes to be used in future genetic improvement of *Ma.* We propose that our findings with the entomopathogen *Ma* will have relevance to improving field performance of other microbial pest-control agents, and thereby reduce dependence on chemical pesticides.

9902657 Hormonal Regulation of Molecular Switches in Molting and Metamorphosis

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Grant 99-35302-8250; $160,000; 2 Years

In insects the periodic production of a new cuticle is necessary for growth and is caused by the hormone ecdysone. At the end of the molt, the old cuticle is shed and the new cuticle hardens. Dopa-decarboxylase (DDC) is a key enzyme for this hardening process. In the larval epidermis of the tobacco hornworm, *Manduca sexta*, as the ecdysteroid titer declines, the ecdysteroid-induced transcription factors MHR3, MHR5, and E75B successively disappear, then bFTZ-F1 appears shortly before DDC RNA synthesis begins. Studies will determine whether particular heterodimers of the ecdysone receptor-ultraspiracle complex and/or MHR5 and E75B are critical for the disappearance of MHR3 as the hormone titer begins to decline and how both MHR5 and E75B are regulated by both ecdysteroid and juvenile hormone (JH). The interactions of these various transcription factors to regulate the proper timing of DDC RNA expression will be studied in the *Manduca* GV1 cell line using both controlled over-expression with a baculovirus promoter and reduced expression by antisense RNA approaches. These studies will increase our basic understanding of the molecular mode of action of both ecdysteroid and Juvenile Hormone mimics which are currently used for control of certain insect pests. They also should provide insight into possible means of interference with the production of DDC and thus cuticular hardening necessary for feeding and survival after the molt. Such information could lead to the development of a new type of insect growth regulator for lepidopteran pests for reduction of their feeding damage to our crops and forests.

9902743 Defense Reactions in Conifers: Oleoresinosis

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Grant 99-35302-4432; $120,000; 2 Years

Bark beetle infestation and associated fungal infection represent the most serious disease problem in conifer species. Conifers defend against attack by the mobilization of stored oleoresin or the induced production of this material at sites of injury. Oleoresin is a mixture of turpentine and rosin, which is toxic to invaders and forms a physical barrier to infection. Manipulation of this natural defense system represents an ideal means of disease control but has yet to be exploited.. The goal of this proposal is to understand this conifer defense mechanism through the study of host systems for constitutive and induced oleoresinosis, and to employ this knowledge to improve disease resistance. The specific objectives of this project include the isolation of additional genes encoding key terpenoid synthases responsible for oleoresin production in firs and pines, the purification of the corresponding recombinant enzymes derived from these genes, and the preparation of antibodies directed against these biosynthetic catalysts. These tools will then be used to determine the cellular organization of constitutive and inducible oleoresin formation in conifers and to define the genetic control of oleoresin formation. Completion of these objectives will allow a comprehensive description of the dynamics, regulation and location of oleoresinosis, and provide the tools and strategies for transgenic manipulation of oleoresin-based defenses. An understanding of oleoresinosis at the molecular level has important implications for bark beetle control in commercial forests.

9902973 The Impact of Generalist Predators in Agricultural and Refuge Habitats

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Postdoctoral Fellowship; Grant 99-35302-8626; $90,000; 2 Years

The goal of this project is to improve conservation, and thus biological control effectiveness, of two important types of predators: ground beetles and wolf spiders. These predators, both of which are native to the United States, occur in a wide variety of crop systems and have been found to control pests. Ground beetles and wolf spiders move into agricultural fields from surrounding, less-disturbed habitats that serve as refuges. Thus the effectiveness of these predators as biological control agents depends in part on their densities in surrounding, non-agricultural habitats. The focus of the proposed research is a field experiment where the immigration rate of ground beetles and wolf spiders will be manipulated to establish vegetable plots with different combinations of these predators. Each field plot will be divided into subplots containing 1) cucumbers or squash and no other plants; 2) three different plant species planted as a refuge for predators; and 3) complex plant communities including many plant species planted as a refuge for predators. The resulting impacts of the predator manipulation on other predators, plant-feeding insects, and plant weights will then be measured, to determine the impact of the ground beetles and wolf spiders in both the agricultural and refuge habitats. Conservation of native predators can increase the effectiveness of biological control. Ground beetles and wolf spiders are common in agricultural fields. More effective conservation of these abundant predators can increase their role in pest control, and thus lessen the need for insecticide applications.

WEED BIOLOGY AND MANAGEMENT

Panel Manager - Dr. Jill Schroeder, New Mexico State University
Program Director - Dr. Ann Lichens-Park

Research grants in this program support studies on the biology and ecology of forest, rangeland, or crop/weed interactions. The ultimate goal is to gain greater understanding of weeds and their relationship with agriculturally important plants, leading to improved integrated and sustainable weed management systems.

Emphasis in this area is on studies that will enhance understanding of how stressful interactions are established and develop between plants; how plants react to stresses generated by such interactions; weed population dynamics; how weed populations adapt to selection pressures, both natural and those imposed by humans; and on the relationship of weed populations to crop, rangeland or forest growth, yield and quality.

9900884 Evaluating Competitive Traits in Rice Cultivars and Potential Improvements in Weed Control
Foin, T.C.; Hill, J.E.; Fischer, A.J.; Gibson, K.D.; Caton, B.P.
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Grant 99-35315-7726; $123,326; 2 Years

Maintaining yield in water-seeded rice requires weed control, which relies heavily on herbicides. The number of herbicides available, however, is being limited by restrictions stemming from environmental and health concerns, by the rapid increase in herbicide resistance, and by the cost and difficulty in bringing new herbicides to market. Growers require weed control methods that will help them conserve their existing herbicides by reducing the rate and frequency of herbicide use. Enhancing weed control by manipulating the rice crop is one means for reducing herbicide use. More competitive cultivars might be developed and used to limit weeds, but a perceived tradeoff between yield potential and competitive ability has inhibited research in this area. Recent research suggests that this tradeoff is not as certain as once believed.

Our long-term goal is to develop rice cultivars permitting reduction in the rate of application of herbicides required for control and the spread of resistant weeds. The first step toward that goal is to identify traits that confer competitive ability in water-seeded rice and their
relationship to yield potential. Understanding which traits are important and when should
reduce the number of genotypes that breeders must screen for selection. We will identify
relevant traits through the integrated use of field experiments and simulation modeling. Our
approach will estimate the potential for weed reduction, measured by decreased weed growth and seed production, in experiments with more competitive cultivars. This research will provide incentives for breeders to begin improving the competitive ability of rice.

9900848 Ecology of *Cynara cardunculus* in Grassland Ecosystems
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Grant 99-35316-7641; $200,000; 3 Years

Cynara cardunculus, artichoke thistle, has invaded thousands of acres of grasslands in California and other regions of the world with a similar Mediterranean climate. Grasslands and rangelands in these regions as well as throughout the western United States are valuable and productive ecosystems that have been impacted heavily by human-caused disturbance, resulting in widespread invasion by exotic plant species. Exotic thistle species constitute a major group of noxious weeds in grasslands throughout the world. As a tap-rooted perennial, *C. cardunculus* is extremely difficult to remove once it is established. Prolific seed production and resprouting from root buds at an early age and following shoot removal compromise control of this weed. The goal of the proposed research is to investigate the mechanism of invasion and subsequent dominance of grasslands by *C. cardunculus*. Four specific questions will be addressed: 1) What site characteristics are conducive to establishment of *C. cardunculus*? 2) What effect does *C. cardunculus* have on a site such that other species are excluded or disadvantaged? 3) What traits contribute to persistence of *C. cardunculus*? 4) What stages in the life history of *C. cardunculus* are most vulnerable to disturbance? Field and laboratory experiments will address the physiological and population ecology of *C. cardunculus* to answer these questions. Results will have direct implications for management of *C. cardunculus* both in the initial stages of spread and in a mature stand. This research will also build on emerging theories regarding the nature of plant invasions.

990886 Reciprocal Weed/Crop Interaction as Influenced by Crop, Planting Date, and Weed Species
VanGessel, M.J.; Sankula, S.
University of Delaware; Department of Plant and Soil Sciences; Newark, DE 199
Strengthening Award; Grant 99-35315-8145; $80,000; 2 Years

The yield reduction caused by a particular weed species depends not only on density, but also on other factors which affect crop and weed growth through time. Manipulating the time of crop planting date alter the impact of weed competition on crop yield, and in turn influence the intensity of weed management to eliminate yield loss. A majority of weed competition studies were conducted with corn or soybean. There has been no attempt to transfer our understanding of this body of yield loss data to less competitive and less acreage crops like vegetables and to determine if a relationship exists for a broader interpretation of existing databases. To bridge this knowledge gap, a study to understand the relationship between planting dates (mid May, mid June, or mid July), crops with different competitiveness (soybean, snap bean, and sweet corn), and weeds of variable growth characteristics (common cocklebur, jimsonweed, and velvetleaf) on reciprocal growth, development, and yield will be conducted at Georgetown, DE. Additionally, weed seed production and seed viability as influenced by planting dates and the appropriateness of transfer of yield loss data from soybean to less competitive snap bean and sweet corn will be evaluated. Information from this research on changes in weed/crop interaction and weed seed production as planting date changes can be fed into the economic threshold models to make cost-effective weed control decisions. Also, this research will derive a significant impact on weed management by decreasing inputs through an understanding of planting time effects on weeds.

9900897 Temperature and Light Regulation of Purple Nutsedge Sprouting and Management
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Strengthening Award; Grant 99-35315-8146; $100,000; 2 Years

Scientists who assessed the world's major weeds have designated purple nutsedge as the world’s worst weed. Dormancy and erratic emergence are key actors in purple nutsedge survival and invasiveness in crops. However, the lack of understanding of these processes makes purple nutsedge management extremely difficult. Apical dominance of tubers in chains (chain dormancy) and diurnally Fluctuating temperatures are probably the most important: factors controlling dormancy and sprouting of purple nutsedge tubers. Therefore, the overall goal of this project is to provide a working knowledge of how the key environmental factors control purple nutsedge tuber sprouting in chains and as individual tubers.

Our previous research showed that breaking chain dormancy and exposure of tubers to diurnally fluctuating temperature can stimulate all tubers to produce sprouts within 3 weeks. Temperature variations regulate both tuber budbreak and bud elongation. However these studies were conducted with one purple nutsedge clone. In order to expand the relevance of our findings, the proposed research will determine whether the fluctuating temperature control of tuber sprouting is a common phenomenon to purple nutsedge ecotypes from diverse geographical sites. We also expect to establish that chain dormancy is mediated by different levels of radiant energy, and that crop canopy can increase chain dormancy and suppress tuber sprouting. An increased understanding of these processes will aid in the development of management strategies to control purple nutsedge and boost crop yields in a sustainable manner.

9900880 Success of Spotted Knapweed: Competition or Site Alteration?
Olson, B.E.; Wraith, J.M.; Hook, P.B.
Montana State University; Department of Animal and Range Sciences; Bozeman, MT 59717
Grant 99-35315-7794; $165,836; 3 Years

Many semiarid grasslands are being invaded by persistent, introduced forbs. Past efforts to restore these grasslands have focused on eradicating these forbs, often with limited success. The lack of success in restoring native species may reflect that invasive forbs are more competitive than native grasses and/or that these forbs alter soil conditions of infested areas, inhibiting germination or survival of native grasses. Our goal is to identify whether the invasive spotted knapweed Centaurea maculosa persists by being more competitive than native grasses, or by altering soil conditions which inhibit the establishment of native species. Competitive ability will be compared by assessing spatial and temporal differences in plant water use and nitrogen (N) uptake between spotted knapweed and two native grasses. Altered site conditions will be identified by assessing short-term effects of these species on soil organic matter and soil N dynamics in the greenhouse, and on soil organic matter and N dynamics, and soil physical/hydraulic properties at three field sites with established populations of spotted knapweed. Effects of altered site conditions will be assessed by monitoring germination of seeds and survival of transplants of the native grasses on infested and noninfested sites. Finally, effects of spotted knapweed litter on seed germination and seedling survival will be assessed in the greenhouse. Results of these studies will be used to identify factor(s) that need to be mitigated before restoring native plants in semiarid grasslands.

9900842 The Physiological Basis for Weed Competition in N2-Fixing Leguminous Crop Systems

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Grant 99-35315-7711; $215,895; 3 Years

Alternative strategies for weed control are needed, to minimize costs for farmers and the environmental problems associated with intensive herbicide use. However, advances require knowledge about physiological mechanisms controlling competition between crop and weed species. In this project, we are attempting to resolve the mechanisms that allow weeds to survive and thrive in low nitrogen conditions in N_2-fixing crop systems. We have preliminary data suggesting that much of the nitrogen in weeds is acquired from the N_2-fixing plants themselves. That possibility will be examined critically using ^{15}N mass spectrometry techniques that allow measurement of nitrogen transfer between the plant types. We also will define the role of soil mycorrhizae, which can connect roots underground and act as 'bridges' for nitrogen transfer. A second focus of the project will be to obtain detailed descriptions of the morphological development of weed and soybean root systems in soil. This will involve the first application of magnetic resonance imaging to weed biology, in an attempt to resolve root structural characteristics that contribute to and control nutrient acquisition efficiency in field soils. Imaging can provide precise estimates of root number, diameter and length, as well as root system architecture at different stages of development. The information generated in the project will be important in developing crop plants with low N transfer rates that minimize weed development with lower herbicide applications.

9900852 The Relationship between Species Richness and Exotic Plant Invasion

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Postdoctoral Fellowship; Grant 99-35315-7695; $76,172; 2 Years

Exotic plant invasions are ubiquitous in the modern agricultural landscape. However, the relationship between biological diversity and invasion by exotic plant species has rarely been tested experimentally. This project will examine invasions by exotic plant species into abandoned agricultural fields to address two basic ecological questions: 1) Does the number of species in a community influence the invasion of exotic plant species into that community? and 2) Does invasion by exotic plant species subsequently affect the number of species in a community? Both of these ideas have been proposed in recent ecological literature, and both may result in the commonly observed negative relationship. The relative importance of these causes and effects of exotic plant invasion must be determined to develop practical management strategies for disturbed lands. This project will answer these questions using two analytical approaches. The first will utilize a long term data set on old field succession at the Hutcheson Memorial Forest Center to examine exotic plant invasions and their effects over a 40 year period. This data set is the longest known continuous study on succession, and provides a unique opportunity to study exotic plant invasions through time. The second approach is an experiment that will use an exotic species addition to specifically test hypotheses about the limiting factors and consequences of exotic plant invasions. Information from this study can be directly applied to many agricultural systems such as grazing, forestry, and crop rotations, to aid in the assessment and management of exotic plant species.

9900846 The Role of Oxidative Stress Tolerance in Crop\Weed Interactions

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Grant 99-35315-7727; $250,000; 3 Years

Weeds compete with crops resulting in costly reductions in both quantity and quality of food and fiber. The development of successful and sustainable weed management systems for today's agriculture requires innovative, integrated approaches based on an understanding of plant physiology, biochemistry, ecology and general agronomy. Oxidative stress is the major factor limiting plant productivity and results from environmental stresses which induce the production of active oxygen species capable of severe cell and tissue damage. The proposed research will measure the amount of oxidative stress imposed during competition between crops and weeds, examine the mechanisms which alleviate oxidative stress in crops and weeds, and determine whether the capacity to relieve oxidative stress is a factor contributing to, the competitive nature of weeds. Cotton and spurred anode will serve as the model species. A comprehensive set of field, pot and laboratory experiments will, 1) demonstrate competition stress between cotton and spurred anode under ultra narrow row compared to conventional production practices, 2) characterize the level of oxidative stress induced by competition, 3) elucidate which particular competitive stress (ie. light, nutrient, water, temperature) is the major contributor to oxidative stress, and 4) determine the oxidative stress tolerance mechanism responsible for crop or weed success. We are evaluating the major tolerance enzymes and antioxidants responsible for plant recovery from oxidative stress. All proposed work will be conducted as a collaborative project between New Mexico State University (NMSU) and USDA-ARS at Stoneville MS. By understanding how plants avoid oxidative stress, crops will be better protected from stress and weeds better managed. In

addition, the gene(s) responsible for oxidative stress tolerance may eventually be identified to improve the competitive abilities of other cotton cultivars or crop species.

9900882 Nitrogen Immobilization for Restoration of Cheatgrass-infested Range

Schupp, E.W.
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Grant 99-35315-7635; $100,959; 3 Years

The goal of my research is to develop a method to restore productivity to degraded weed-infested rangelands using native disturbance-adapted species to suppress weeds and promote succession to native rangelands. In this renewal I study the potential of nitrogen immobilization (reducing nitrogen availability to plants by stimulating microbial growth) to reduce negative effects of annual weeds and increase establishment of native perennials using as a model system the weed cheatgrass and the native early seral grass bottlebrush squirreltail. Specific objectives are to evaluate (1) whether nitrogen manipulation alters competition between cheatgrass and squirreltail during seedling establishment and (2) whether weed seedling emergence is more sensitive to nitrogen manipulation than is native perennial emergence, and (3) to continue monitoring growth and survival of squirreltail and bluebunch wheatgrass established in my present experiment. For objective 1) I use 2-species mixed density plots in greenhouse and field experiments with 3 nitrogen treatments including Nitrogen-immobilization and Nitrogen-addition, and develop models that quantity plant responses (biomass, survival, and reproduction) to mixed densities. For objective 2) 1 use greenhouse and field experiments to evaluate nitrogen effects on seedling emergence and establishment. for objective 3) I monitor already established perennials and their effects on weeds. The overall strategy may substantially benefit agriculture by improving productivity of degraded rangelands and facilitating their sustainable use. By using native species and promoting natural succession to restore rangelands I focus on a novel pest management strategy and on sustainable weed management practices.

BIOLOGICALLY BASED PEST MANAGEMENT

Panel Manager - Dr. Donald Cooksey, University of California, Riverside
Program Director - Dr. Mary Purcell

Research grants in this area support mission-oriented studies to develop methods of integrated pest management. The term pests includes weeds, arthropods, plant parasitic nematodes and plant pathogens. Systems under study include pests in horticultural and field crops, forests, rangelands, urban landscapes, food or feed transported and stored for human or livestock consumption. The program supports both biological control and other research aimed toward developing and integrating management practices into an overall system with the goal of reducing dependency on synthetic pesticides.

Research areas include but are not limited to: 1) factors that influence effectiveness of biological control, 2) mass production and implementation methods for biological control organisms, 3) factors that conserve or enhance biological control habitats, 4) systematic, taxonomic, or biogeographical studies from which discoveries of new biological control agents can be made, 5) assessment of conventional and alternative pest management practices, 6) development of disease warning systems, 7) resistance management studies, and 8) use of pheromones.

9902145 Integrated Biological Control of Tomato Viruses and Nematodes
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Auburn University; Department of Plant Pathology; Auburn, AL 36849
Grant 99-35316-8006; $168,338; 2 Years.

The project involves integrating two tactics, which independently have demonstrated plant disease control potential, into a management system for nematodes and viruses of tomato. The tactics are 1) strains of plant growth-promoting rhizobacteria (PGPR) and 2) chitin as a model organic amendment. The PGPR are spore-forming bacilli, which can be readily formulated in a stable manner. The target pathogens, viruses and nematodes, are two of the most difficult pathogens to manage by biological means. Amendments of soil with chitin have been shown to manage plant parasitic nematodes partially by stimulating an antagonistic microflora. PGPR have demonstrated some capacity to reduce nematode damage, and in combination with chitin, we expect the effects to be enhanced. Some of the PGPR strains have demonstrated induced resistance activity, whereby the host plant's defenses are activated, and this has led to reductions in severity of cucumber mosaic virus on tomato. Additionally, the combination of PGPR and chitin results in marked promotion of plant growth, allowing us to test the hypothesis that tomato plants exhibiting promoted growth will be more resistant to damage by viruses. Information from the project will help with two national priorities-assessments of alternatives to methyl bromide for soil fumigation and management systems for whiteflies which are vectors of geminiviruses.

9902197 Development of Integrated Systems of Induced Resistance in Cucurbits
Kloepper, J.W.; Zehnder, G.W
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Grant 99-35316-8006; $151,930; 2 Years

Diseases and insect pests severely impact vegetable production in the southeastern U.S. With cucumber, for example, in any field season multiple disease and insect pests simultaneously threaten plant growth and yield. Pest management by traditional means requires use of both fungicides and insecticides over several seasons. An alternative management approach is activation of plant resistance. Specific biotic and abiotic agents are known to induce resistance against disease and insect pests, and it has also been shown that wounding of plants by insects can induce resistance against attack by other insects. The process of induced resistance involves the activation of plant defense genes and production of a host of plant defense compounds. Recent evidence has shown that specific elicitors or inducing agents activate different plant metabolic pathways. However, no studies have been done to determine the compatibility of resistance induced by chemical and biological elicitors, or to evaluate the influence of insect feeding damage on induced resistance following treatment by inducing agents. This project will be done to evaluate the compatibility of commercially available chemical and biological inducers for resistance against multiple diseases in cucumber in the presence and absence of insect feeding damage. The results will aid in the development of recommendations for optimal use of induced resistance technology in pest management programs.

9902187 Optimizing and Validating a Novel Mark-Release-Recapture Method
Hagler, J.R.; Jackson, C.G.; Blackmer, J.L.; Gould, J.R.; Henneberry, T.J.
USDA Agricultural Research Service; Western Cotton Research Laboratory; Phoenix, AZ 85040
Grant 99-35316-7955; $143,167; 3 Years

New techniques in the study of insect dispersal may significantly decrease the need for chemical pesticides and the use of hazardous marking agents. Our new immunological marking technique is faster, cheaper, and more effective than current insect markers. Tiny (microscopic) beneficial insects sprayed or fed vertebrate proteins prior to release can be recaptured and identified using antibody testing. Methods similar to medical laboratory procedures used to test for

pregnancy or disease enable us to distinguish released insects from native insects. Small parasitoids could be used to biologically control pest insects such as silverleaf whitefly. Using the immunological markers we will study the within-and between-crop dispersal patterns of a small parasitoid of the silverleaf whitefly, a major crop pest. The major goals of our research are to: validate the use of protein markers for dispersal studies, quantify parasitoid dispersal, and determine what factors influence parasitoid dispersal (e.g., wind direction, wind speed, temperature, humidity, time of day, cropping system, etc.). Information from these studies will help us to understand parasitoid behavior so we can better manage them to biologically control plant pests such as the silverleaf whitefly.

9902156 Field Release of Phorid Flies for Classical Biocontrol of Imported Fire Ants

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Grant 99-35316-7849; $241,756; 2 Years

Imported fire ants are one of the most abundant pests in the southeastern United States and they have recently spread into California. With densities of 1,500 ants per square yard, these pests are ubiquitous in parks, pastures, yards, and cultivated fields. These ants are responsible for billions of dollars in damage to agriculture, human health, and the environment. Fire ant densities in the United States are about five times higher than those in South America. Escape of the ants from natural enemies left behind in South America is a likely explanation for this difference. Phorid flies in the genus *Pseudacteon* are a promising group for biological control of imported fire ants because: 1) they are highly host specific, 2) they are broadly distributed, and 3) they have demonstrated effects on fire ant populations. We will release phorid flies in 12 areas around Gainesville, FL. Each area will consist of paired treatment and control sites where we will monitor fire ant population densities. We will also monitor fly populations and their rates of dispersal. Results of these studies will allow us to determine how effective these flies are at suppressing fire ant populations and how many release sites will be necessary to achieve results in a specified time period. Successful releases of these flies and other natural enemies will not eradicate imported fire ants, but they could help tilt the ecological balance in favor of our native ants. If this happens, imported fire ant populations here could drop to levels similar to those in South America.

9902195 Conservation Biological Control in Ornamental Landscapes

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Grant 99-35316-7850; $158,404; 2 Years

Americans spend an estimated one billion dollars annually on maintaining plants in ornamental landscapes. Many of these dollars go to controlling insects that feed on plants. These pests are favored in man-made habitats, such as agricultural cropping systems and ornamental landscapes, that do not provide a suitable environment for the natural enemies (predaceous and parasitic insects) that typically regulate insect populations. Ornamental landscapes are much more hospitable environments for natural enemies than agricultural fields because ecological disturbance may be minimized once plantings have been established. It has long been suggested that natural enemies may be encouraged in landscapes by planting greater numbers and higher diversities of flowering plants that provide them food and refuge. Our research project tests the hypothesis that inclusion of flowering plants in ornamental landscapes encourages natural enemies of insect pests. We will construct 42 identical study plots in each of two sites, each plot planted with a pine tree (at the Illinois site) or a euonymus plant (at the Indiana site) that is infested with plant-feeding insects. Within these plots, we will vary the density of four species of flowering plants that provide nectar and pollen for natural enemies, and will subsequently sample plots at intervals over a two-year period to estimate the abundance of natural enemies, and the mortality rates of pest insects. This study will allow us to determine whether flowering plants do indeed foster natural enemies and encourage suppression of plant-feeding pests.

9902176 The Efficacy and Economics of Stored Food Corn Ozonation to Maintain Grain Quality

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Grant 99-35316-8656; $158,394; 2 Years

The goal of this research is to optimize existing ozone fumigation parameters both technically and economically. In the long term this is expected to benefit U. S. food grain producers, handlers, and processors with potential savings of millions of dollars from unnecessary pest control treatments, improved grain quality, additional export opportunities, and safer cereal-based foods. It will provide consumers with grain that is free of pesticide residues and fungal toxins exceeding current and future limits, is reasonably priced, is pest and filth free, and has been preserved as a nutritionally wholesome product. Specifically, this project proposes to take our existing ozone research to the final phase, examining the economics of ozonation, determining optimal fumigation parameters, the influence of ozonation on end-use qualities, and the application of ozone to the head space as a preventative pest management tool. The contamination of grain with insects, insect fragments, mycotoxins and pesticide residues is a major concern of the U.S. grain producing and food processing

industry. In the past, synthetic pesticides have been used to achieve therapeutic control. However, insects are developing resistance, consumers are concerned about pesticide residues and toxins, and many pesticides are being withdrawn from use. Therefore, alternative, preferably non-residual chemical methods of pest control are needed. Ozonation of food grains is a newly emerging pest management technology for stored food grains. The benefits of ozone fumigation research to the U. S. grain industry in terms of reduced dependence on residual chemicals, and to the consumer in terms of increased food safety, will be significant.

9902169 Pollen and Phytoseiid Diet Specialization in Biological Control of Thrips

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Grant 99-35316-7939; $122,186; 2 Years

Western flower thrips is the most important insect pest of greenhouse flower and bedding plant crops in the U.S. These crops are a large and expanding part of American agriculture, especially in urbanized states. This pest is difficult to control with pesticides because it hides in plant buds and is resistant to many pesticides. Biological control of this pest would increase profits by reducing losses and reduce pesticide exposure to workers. Current biological control agents provide partial control of thrips on flower crops. New research is needed to enhance this control to commercial levels. Predatory mites (Phytoseiidae) are the most promising, commercially available group of thrips natural enemies. These mites also feed on pollen, although the importance of pollen varies among species from slight to critical. This project will examine how species of mites that vary in their degree of pollen feeding respond when greenhouse crops are treated weekly with suitable pollen. The goal of the grant is to locate the optimal species (of 8 to be tested) that responds most strongly to pollen applications. The effects on pollen supplements on increase in total numbers of mites over time and their total feeding on thrips will be determined in replicated, whole greenhouse experiments. This information will be useful to commercial insectaries because it will identify the best mite species to rear and the degree to which pollen applications improve thrips control. This information will allow insectaries and extension agents to provide guidance to growers about use of mites for biological control of thrips.

9902394 Evaluating Indirect Ecological Effects of Biological Control: A Symposium Workshop

Hoelmer, K.A., Quimby, P.C.; Kirk, A.A.; Villet, R.
USDA Agricultural Research Service; Beltsville Agricultural Research Center; Beltsville, MD 20705
Grant 99-35316-7912; $5,000; 1 Year.

Biological control is regarded as one of the most environmentally sound methods of pest management, and is increasingly the option chosen whenever possible. However, biological control has been subjected to increasing scrutiny from public interest groups, government agencies and the scientific community, as attention has been focused on the consequences of introduced exotic organisms. Federal policy requires that the impact of biological control programs on non-target organisms, as well non-target pests, be evaluated and optimized. There has been contentious discussion in recent years regarding indirect effects of biological control. This conference will be unique in gathering leading international ecologists and biological control scientists to develop guidelines for improving the science of biological control. Because substantial modern research in ecology, evolutionary biology and theory and practice of biological control has occurred outside of the United States, the international conference in Montpellier, France, will significantly influence future biological control programs in the U. S.

The conference will be held at Agropolis in Montpellier, France, the site of the USDA-ARS European Biological Control Laboratory. Overviews presented by ecologists and biological control scientists will critically assess the status of evaluation efforts. The workshop will then identify and recommend specific ecological approaches for improving biocontrol projects. Sponsors include the International Organization of Biological Control, AGROPOLIS (an international agricultural research and education complex) and the International Centre for Biological Control AGROPOLIS (including the USDA-ARS European Biological Control Laboratory). Subventions have been obtained from U.S. and French organizations.

9902168 Can Refuges Moderate Disturbance and Enhance Biological Control in Annual Crops?

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Michigan State University; Department of Entomology; East Lansing, MI 48824
Grant 99-35316-7911; $162,161; 2 Years

Annual crops form the backbone of U.S. agriculture but have proven to be difficult environments in which to implement biological control. At the heart of this difficulty is the frequency and intensity of the disturbances imposed on these systems. Annual crop fields typically undergo multiple and intense disturbances (tillage, fertilizer and pesticide applications, cultivation, harvest, etc.) which limit their suitability for supporting effective populations of natural enemies. We propose that to improve biological control in annual crop systems it is necessary to understand how natural enemies are impacted by disturbance and to modify disturbance regimes to conserve these biological controls. The USDA National Conservation Buffer Initiative represents a great opportunity to manage the ecological infrastructure of agricultural

landscapes to benefit soil and water quality, improve wildlife habitat and enhance biological control. Conservation buffers are areas of vegetation established along streams, ditches, field borders within fields, to reduce soil erosion and intercept chemical runoff. Ground beetles (Coleoptera: Carabidae) are a well-known and important group of predators that consume both insect prey and weed seeds. The overall goal of our research is to understand how stable habitats such as conservation buffers (i.e. refuges) interact with highly disturbed annual crops in determining the dispersal, diversity, and abundance of ground beetles. Secondly, we will evaluate the role of refuges in moderating the impact of disturbance on carabid communities. Finally, we will suggest techniques for managing refuges to improve biological control of insect and weeds.

9902151 Intercropping as a Disease Management Tool for Early Leaf Spot of Peanut

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Strengthening Award; Grant 99-35316-8007; $90,000; 2 Years

Early leaf spot of peanuts (ELS), caused by the fungus *Cercospora arachidicola, is* a devastating disease worldwide, with few control options available. Intercropping, the cultivation of multiple crop species in close association often employed in subsistence agriculture, will be evaluated for its potential as a non-chemical, biologically-based disease management strategy in a modern industrialized production setting. Peanuts inter-cropped with corn will be our model system, but the approach engendered here could be applied to numerous and diverse other systems, both large-scale mechanized and small-scale manual, in the U. S. and abroad. This goal will be accomplished through three specific objectives. First, we will quantify ELS incidence and crop yield throughout two seasons in field plots containing peanut monocrops, intimate intercrops (alternating rows of corn and peanuts), and strip intercrops (4 rows corn alternating with 4 rows peanuts). Second, we will compare disease dispersal patterns among these treatments. Third, we will evaluate the impact of proximity to corn on disease development after dispersal, particularly as influenced by changes in microclimate, at a separate location. The first goal has immediate application; the second goal will improve our understanding of the mechanisms at work and help generalize to other crop-disease systems. Preliminary data suggests that corn can reduce ELS severity on peanuts. If compatible with mechanization, intercropping could be readily adopted by many farmers as a low-input cultural control in this case and others, particularly where few alternatives to chemical disease management exist. This will enhance the long-term sustainability of U. S. agriculture.

9902179 Seed Exudate Fatty Acids: Host Plant Factors Regulating the Biological Control Efficacy of Seed-Applied Rhizobacteria

Nelson, E.B.
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Grant 99-35316-7910; $129,009; 2 Years

Biological disease control, which relies on the deployment of microorganisms to control plant diseases, represents an approach to disease management that can reduce the dependency of farmers on chemical fungicides for the maintenance of healthy crops. One of the greatest obstacles to the implementation of these practices in U.S. agriculture has been the variable and unpredictable performance of introduced microorganisms. This research is aimed at improving our understanding of how crop plants, primarily through the properties of materials released from their seeds, regulate biological control processes. The main goal of our research is to understand how the introduced bacterium, *Enterobacter cloacae,* suppresses seed and seedling rots caused by the oomycete *Pythium ultimum* on some plants but not others. From our preliminary research, we theorize that fatty acids released from seeds during germination regulate this activity. In this research we will determine the types and amounts of fatty acids released during germination from seeds of plants that differ in disease susceptibility and ability to support disease control by *E. cloacae.* We will also assess the impact of seed treatment with *E. cloacae* strains on fatty acid release characteristics from seeds and evaluate how sugars that are also released from seeds during germination affect the ability of *E. cloacae* to metabolize fatty acids and suppress *Pythium* responses to seeds. Our longer-term goal is to use this information to predict performance of biological control organisms in association with different crop species and to develop more effective methods for the successful implementation of biological control systems in crop production.

9902150 Development of a Forecasting Model to Optimize Biological Control of Plant Disease

Johnson, K.B.; Stockwell, V.O.
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Grant 99-35316-7954; $133,245; 2 Years

Variation in the efficacy of biological agents for control of plant disease has been an impediment to the wider use of this management strategy in commercial agriculture. Among the components of this variation, the direct effects of environment on the establishment, growth, and persistence of biological agents is one of the most significant, particularly at the point of implementation when the technology passes from experienced developers to inexperienced users. In this proposal, we will evaluate the effects of temperature and potential competition from indigenous microbes on the growth and

persistence of two bacterial agents that suppress fire blight disease of apple and pear. These agents suppress fire blight when they are sprayed on and colonize the surfaces of apple and pear blossoms prior to the arrival of the fire blight pathogen. Preliminary data indicate that orchard temperatures following application of the bacterial agents contribute to their effectiveness. Temperature also is an important indicator of fire blight risk. To fully integrate biologically-based methods with conventional management, models are needed that will optimize the use of biological agents. In this proposal, we will develop a model that will identify periods favorable for the introduction of bacterial biocontrol agents into orchard environments. Through use of extended weather forecasts and a regional, internet-based delivery system, this model will provide the lead-time required to establish bacterial agents in blossoms prior to a period of high disease risk.

9902194 Impacts of a Transgenic Insecticidal Crop on Parasitoid Fitness and Biological Control Performance

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New Investigator Award; Grant 99-35316-7913; $236,370; 2 Years

Transgenic insecticidal crop cultivars (TICs) are cultivars that are genetically engineered to express insecticidal activity against pests. They are predicted to become a major insect management tactic in the next century. Current research on TICs focuses largely on the evolution of pest populations that are resistant to these cultivars, while the potential environmental impacts of TICs have not been examined, including their impact on naturally-occurring biological pest control within and outside transgenic crops. Naturally-occurring biological control relies on a pest's existing natural enemies within a transgenic crop, and is the most important component of integrated pest management (IPM) systems. Moreover, naturally-occurring, biological control relies on the movement of natural enemies between crops; natural enemies destroyed within a transgenic crop cannot provide biological control in other crops. Our research will be the first to examine the potential impacts of a TIC on the performance of parasitoids as natural enemies, and on naturally occurring biological control within and without a transgenic crop: Parasitoids often are the most important natural enemies of pests. The model for this research includes: transgenic and non-transgenic sugarcane, non-transgenic corn, Mexican rice borer (the TIC's target pest), sugarcane borer (a non-target pest under biological control), and *Allorhogas pyralophagus* and *Cotesia favipes* (parasitoids of these pests in sugarcane and corn crops). Our research will, *i*) contribute to the long-term improvement and competitiveness of U. S. agriculture by improving the sustainability of TICs and by understanding their impacts on naturally-occurring biological control, *ii*) advance our understanding of biological control and, *iii*) foster the use of these technologies as components of IPM strategies that do not rely on chemical insecticides.

9902186 Genetic Improvement of Biocontrol Agents to Manage Soil-borne Plant Pathogens

Kenerley, C.; Howell, C.
Texas A&M University; Department of Plant Pathology and Microbiology; College Station, TX 77843

Grant 99-35316-7940; $262,201; 3 Years

The application of biological agents to manage pathogens that attack crop plants is a vital component of biointensive Integrated Pest Management. The greatest impact biological agents will have is on the reduction of pesticide usage for management of Soil-borne plant diseases. This project will use a molecular approach to manipulate selected genes in the biocontrol agent, *Trichoderma virens.* These genes encode for hydrolytic enzymes that are involved in the degradation of cell walls of plant pathogens. The goal is to provide an understanding of what genes are most important in the biological control of seedling pathogens and to produce superior biocontrol strains of *T. virens.* The importance of each selected gene will be determined by constructing strains of *T. virens* that lack the gene or have multiple copies of the gene. Each of these strains will be assessed for many traits including the ability to grow on roots, produce hydrolytic enzymes, protect seedlings from pathogen attack and induce plant defense compounds. After individual genes have been assessed, biocontrol strains will be generated that contain multiple copies of pair-wise combinations of the genes. The synergistic effect of these gene combinations for controlling seedling disease will be determined. The most effective gene combinations will be placed into other strains of *T. virens* that produce different antibiotics involved in biocontrol to prolong the usefulness of the selected genes. This research will demonstrate genes and gene combinations that can be used to enhance the activity of biological agents for the control of seedling diseases in an environmentally acceptable manner.

9902175 Thermal Mortality of Insects and Post-harvest Control of Insect Pests in Nuts

Tang J.; Cavalieri, R.; Swanson, B.
Washington State University; Department of Biological Systems Engineering; Pullman, WA 99164-6120

Grant 99-35316-8099; $148,514; 2 Years

Alternative phytosanitation and quarantine treatments are needed for domestic and international markets. New thermal treatments are being investigated because of the uncertain future of other chemical fumigants and increasing public concern over residues in foods. For successful development of those treatments, a delicate balance must be determined between killing insects and minimizing thermal impact on product quality. Our objectives are to study the kinetics for

thermal mortality of codling moths, navel orangeworms and Indian meal moths, and to develop practical phytosanitation and quarantine treatments for walnuts and almonds using radio frequency (RF) and microwave energy. We will use an innovative heating block system, developed at Washington State University, to measure mortality of the insects at selected temperatures in ambient and controlled atmospheres. We will determine temperature-time combinations to kill insects in their most heat-resistant growth stage. This information will be used to develop effective RF and microwave treatments based on mortality data, heat transfer and electromagnetic theories and food quality. This research will establish a sound basis for developing thermal control methods for insect pests and benefit other researchers working on alternative thermal treatments. The proposed research will help to develop an effective and environmentally sound insect pest control process for nuts and fruits, in order to maintain a viable, competitive U.S. nut and fruit industry in the global market.

PLANTS

Fundamental and mission-oriented research is supported that provides basic understanding of mechanisms and processes of plant systems. This knowledge is necessary as a basis for developing management strategies for improving and sustaining agricultural production systems. Grants are awarded under five programs: Plant Genome, Plant Genetic Mechanisms, Plant Growth and Development, Photosynthesis and Respiration, and Nitrogen Fixation and Nitrogen Metabolism.

PLANT GENOME

Panel Manager - Dr. Jeff Bennetzen, Purdue University
Division Director - Dr. Ed Kaleikau

This competitive grant program is part of the National Plant Genome Initiative (NPGI) coordinated by the National Science and Technology Council (NSTC)--the long-term goal of this interagency effort is to understand the structure and function of genes in plants important to agriculture, environmental management, energy and health. The NRI participates in the NPGI and to the mission of agriculture to promote sustainability and profitability of plant production, and to improve the quality of food, fiber, and feed. To accomplish these goals, the NRI Program gives high priority to research for construction of genomic maps, and for detailed studies of specific regions of genomes, genes, or genetic processes. The program also supports research on development of new methods or innovative approaches that have potential application to create new germplasm/varieties, genome mapping, gene isolation, or gene transfer in crop and forest species as well as bioinformatics.

9901397 Enhancing Recombination and Introgression between Related Genomes via Suppression of the Mismatch Repair Gene MSH2

Chetelat, R.T.; Britt, A.B.
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Grant 99-33500-7683; $260,000; 3 Years

Wild relatives of crop plants are an important source of genes for disease and insect resistance, yield, fruit size, and environmental stress tolerance. Genes in wild species may not be accessible due to hybrid sterility, caused by the failure of chromosomes to pair and exchange genetic information (recombine) normally. When recombination is suppressed, large tracts of DNA on either side of a gene of interest are transferred, resulting in reduced yield, fruit size, etc. Genes involved in the repair of mistakes in DNA sequences (mismatch repair genes) help determine the rate of recombination. When one of these genes (MSH2) is turned off in yeast, recombination between related species is increased. We have cloned an MSH2 gene from tomato (*Lycopersicon esculentum*), an important crop plant in the U.S. (worth over $1.8 billion annually) and a source of vitamins and antioxidants. We will generate tomato plants with low levels of MSH2 gene expression, and cross them to the wild nightshade *Solanum lycopersicoides*, whose chromosomes do not pair or recombine at a normal rate with those of tomato, producing sterile hybrids. We will study the impact of MSH2 expression on fertility, pairing, and recombination in these hybrids and other genetic stocks. If successful, this project should provide a broadly applicable method to aid the genetic improvement of other crop plants by gene transfer from wild relatives.

9600856 Positional Cloning of the Rice Submergence Tolerance Gene Sub1

Mackill, D.J.; Ronald, P.C.
USDA Agricultural Research Service; Crops Pathology and Genetics Unit; Davis, CA 95616
Grant 99-35300-7751; $230,000; 3 Years

Flooding is a major constraint to crop production in the U.S. and causes enormous losses during periods of heavy rainfall or when rivers overflow. While rice is well suited to growing in submerged soils, too much water can damage or kill the plants. Most rice varieties are killed by less than a week of submergence, but a few tropical strains have the ability to tolerate complete submergence for up to two weeks. We have recently identified a gene in rice, designated *Sub1*, that confers this tolerance. This gene has been located on the rice genetic map by the use of DNA markers. The present project aims to isolate this gene through the methods of "map-based cloning." Once the gene is isolated, it can be transferred into other rice cultivars and other crop species. This will provide valuable insights on the response of plants to flooding, and on how flooding tolerance can be increased. In rice, this gene could be useful in enabling U.S. rice varieties to withstand prolonged submergence. This would allow the use of deeper water levels during seeding, thereby increasing weed suppression and reducing the need for herbicides. In other species, this gene will provide valuable information on the mechanisms of flood resistance. It may also directly increase the level of flood tolerance when introduced into crops that are highly sensitive to flooding.

9901317 Comparative Genomics of Lettuce and Sunflower

Michelmore, R.; Knapp, S.J.
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Grant 99-35300-7739; $240,000; 2 Years

A global consideration of plant genomes is required to allow genetic and functional inferences to be made between agriculturally-important and model species. Lettuce and sunflower are two economically important representatives of the Compositae which is one of the biggest and most ecologically-successful plant families. We will develop a partial genetic map, based on co-linearity of genes (syntenic map) of lettuce and sunflower. This will leverage genomic information from model organisms, particularly *Arabidopsis*, as well as contribute genetic, physiological, and phenotypic information to the

general syntenic map that will develop for plants. We aim to map approximately 400 new sequenced-based markers in each of lettuce and sunflower using existing, well-characterized, single populations of lettuce and sunflower. All mapped genes will be sequenced, and orthologous (corresponding) positions identified in *Arabidopsis* to determine synteny within the Compositae and between the Compositae and *Arabidopsis*. By combining our efforts at the University of California, Davis and Oregon State University, Corvallis, we will considerably increase the efficiency and reduce the cost of investigating synteny in these species. The information will be generated as the sequencing of the *Arabidopsis* genome is completed. Ultimately, the map will include candidates for agriculturally useful genes that have been integrated with PCR-based markers suitable for breeding purposes.

9901333 Mapping, BAC Recovery, and Positional Cloning of Loci Involved in Fertility Restoration

Pring, D.R.; Klein, R.R.
USDA Agricultural Research Service; Crop Genetics and Environment Research Unit; Gainesville, FL 32604
Grant 99-35300-7725; $200,000; 2 Years

Hybrid grain sorghum production in the U.S. is dependent on the use of cytoplasmic male sterility (cms), which allows efficient production of hybrid seed. Nearly all hybrid sorghum seed production relies on a single source of cms, resulting in genetic uniformity. Such uniformity generates potential vulnerability to pests, resulting in disease epidemics such as occurred in corn. We have described the biology and genetics of an alternative source of cms in sorghum. This source of cms, from the line IS1112C, confers male sterility in a wide variety of germplasm because genes that restore male fertility are rare. We have traced the possible causal DNA sequence to the mitochondrion, the energy-producing organelle of the cell. Two genes are required to restore fertility. We propose to develop molecular markers for these genes through an approach described as bulk segregant analysis and amplified fragment length polymorphism. These markers will allow us to rapidly incorporate the two genes into important germplasm. The markers will be placed on the genetic and physical maps of the sorghum genome, and used to isolate bacterial artificial chromosomes that may carry the genes. A technology designated gene capture will be employed to recover DNA sequences of the genes. If successful we will be able to fully describe the nature, and the possible mode of action, of genes that restore male fertility in sorghum, and determine how they interact with the cms-causing genes of the mitochondrion. Such studies allow understanding of the basis of traits important to plant reproduction.

9901330 Cytomolecular Mapping in Sorghum and Maize Using In Situ Hybridization

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Postdoctoral Fellowship; Grant 99-35300-7819; $90,000; 2 Years

The activity of a gene is determined not only by its DNA sequence, but also by its location on a chromosome. Consequently, an understanding of chromosome structure is prerequisite to understanding how genes function in living organisms. The most direct means by which genes can be positioned on chromosomes is by fluorescence in situ hybridization (FISH). In FISH a DNA marker associated with a gene is hybridized to chromosomes fixed on glass microscope slides. The chromosomal location of the marker (gene) is then visualized. We propose to use FISH to position markers at various places along a specific sorghum chromosome. The resulting “cytomolecular map” will be compared with the existing genetic map of this sorghum chromosome. If time permits, DNA markers used in constructing the sorghum cytomolecular map will be hybridized to maize chromosomes to construct cytomolecular maps for maize chromosomal regions sharing homology with the sorghum markers. This work will contribute directly to long-range improvement and sustainability of U.S. agriculture by providing (1) insight into the relationship between linkage distances, base pair distances, and chromosome structure in two important crop species, (2) a better understanding of the cytological and molecular factors affecting recombination, a process that is central to plant improvement, (3) means by which the site of a transgene insertion can be rapidly detected and characterized, (4) a better understanding of the evolutionary divergence of Sorghum and Zea, and (5) a foundation for further cytomolecular mapping in other crops.

9901316 Physical Mapping and Microcollinearity of Apospory-specific Regions in Two Grasses

Ozias-Akins, P.; Roche, D.; Hanna, W.W.
University of Georgia; Horticulture Department/USDA Agricultural Research Service; Coastal Plains Experiment Station; Tifton, GA 31793
Grant 99-35300-7691; $300,000; 3 Years

Several plant species are able by natural means to produce clones of themselves through seeds. This genetically determined potential is called apomixis. We are studying a form of apomixis called apospory which occurs most frequently in the grass, rose and daisy families. Although rare in crop plants, this ability to reproduce asexually through seeds would provide a tremendous breeding tool, thus transfer of apomixis to crops is a long-term goal of ours. It would revolutionize the way we breed new cultivars and produce seeds especially for crops such as wheat, corn, barley, rice,

sorghum and millet. Breeding cycles could be shortened and hybrids more easily produced. Considering the far-reaching potential impact on grass and grain crops we study apospory in two grass species, *Pennisetum squamulatum* and buffelgrass (*Cenchrus ciliaris*), both of African origin, with the hope to transfer their natural reproductive trait to crops. We have genetically defined a genomic region responsible for the apomictic mode of reproduction which is remarkably conserved between these two species. In the proposed work we intend to determine the position of that region within the whole genome and to compare its fine structure between the two species. DNA segments which are highly conserved between the two species are more likely to contain genes of interest than those segments which are greatly divergent in DNA sequence. It is on these conserved segments that the gene(s) for apomixis will be characterized eventually.

9901401 A Survey of Microsatellite Variation in U.S. Corn Belt Germplasm

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Grant 99-35300-7757; $340,000; 3 Years

Genetic variation or diversity is the raw material of selection and forms the basis for future improvements in the quality and productivity of our agricultural crops. Little is know in corn about the amount of genetic diversity currently present or how it has changed over time. Most hybrids grown by farmers today were developed from a race of maize called the Corn Belt Dents. The Corn Belt Dents were widely grown by U.S. farmers prior to the introduction of hybrids in the 1930s. We propose to use DNA markers to assess the amount of genetic variation that was present in a sample of 600 accessions of Corn Belt Dents, their progenitor races (Northern Flints and Southern Dents), and Corn Belt Dent derivatives (commercial hybrids, public inbreds, etc.) by sampling these groups broadly and by genetically describing the relationships of the various types to each other with the DNA markers. This information will have a direct impact on corn breeding, by providing breeders with previously unavailable information, provide information on the selection history of the Corn Belt Dents, and contribute to an understanding of the conservation of Corn Belt Germplasm. This study is relevant to long-range improvements and sustainability of U.S. agriculture by providing much needed data about the current status of genetic diversity in corn.

9901334 Genomic and Physical Organization of Fertility Restorer Genes in *cms-T* Maize

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Grant 99-35300-7752; $330,000; 3 Years

Cytoplasmic male sterility (CMS) is a maternally inherited inability to produce viable pollen. Because CMS eliminates the need for removal of the male flowers prior to hybridization, the CMS trait is invaluable in plant-breeding systems. CMS systems are found in over 150 plant species and are usually attributed to novel mitochondrial genes. These genes encode aberrant proteins that often interfere with normal mitochondrial function and pollen development. CMS-associated, mitochondrial anomalies can be suppressed by the action of nuclear *Rf* or *Fr* genes, whereby male fertility is restored. In many instances, *Rf*-gene-mediated fertility restoration is associated with the modification of CMS-associated, mitochondrial RNAs. This phenomenon has been observed in maize, sorghum, oilseed rape, rice, wheat, sunflower, petunia, and common bean. In many of these economically important plants, CMS is the only efficient way to produce hybrid seed. We will physically characterize the genomic region spanning *Rf1*, one of the two complementary restorers for T-cytoplasm maize. We will also clone and characterize additional mutant alleles of *Rf1* via AFLP-transposon display. Finally, we will analyze the expression of candidate *Rf1* sequences via complementation in our newly developed T-cytoplasm transformation system. Completion of this project will provide a physical foundation for the investigation of nuclear-mitochondrial interaction and RNA processing in plant systems. Because CMS remains a widely used tool for the production of hybrids in a number of economically important crops, we anticipate that a common mechanism among *Rf1*-like restorers will be applicable to these crops as well.

9901400 Genetic Mapping and Introgression of Yield Genes into Soybean from *Glycine soja*

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Grant 99-35300-7820; $300,000; 3 Years

The world demand for soybean is expected to grow and one of the most cost-effective ways for improving the production efficiency of the crop is by genetically increasing the yield of cultivars. Unfortunately, there is limited genetic diversity in soybean that can be used by plant breeders in improving the crop. The goal of our project is to increase soybean yield through identifying the locations of yield improving genes from *Glycine soja*. *G. soja* grows wild in eastern Asia and is believed to be the ancestor of soybean. Similar research on tomato and rice has resulted in the identification of yield improving genes from wild relatives. Our research will be done using populations developed by making several crosses

between a *G. soja* line and a soybean variety. These populations will be evaluated for yield and other traits in field trials in Illinois and Nebraska during 1999 and 2000. The populations also will be evaluated with DNA markers in the laboratory. The field test and DNA marker data will be analyzed to determine the chromosomal locations of genes that control yield and other traits. If the locations of genes from *G. soja* that improve yield are identified, a second set of populations will be evaluated to confirm the effect of the mapped genes in additional environments and genetic backgrounds. If we confirm the presence of yield improving genes from *G. soja*, these genes will be rapidly moved into soybean varieties grown by farmers.

9901415 Isolation of Bacterial Blight Resistance Genes from Soybean

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Grant 99-35300-7693; $180,000; 3 Years

We are investigating the molecular basis of disease resistance gene specificity in plants. The effectiveness of naturally occurring disease resistance genes is often limited by their extreme specificity. A single resistance gene is typically effective against only specific pathogen strains. However, greater use of naturally occurring disease resistance genes is dictated by a lack of environmentally sound pesticides. The knowledge gained from our studies could lead to engineering of disease resistance genes with broader specificity. As a first step towards understanding specificity, we have been comparing disease resistance genes in soybean and *Arabidopsis* that confer resistance to bacterial blight disease, which is caused by *Pseudomonas syringae*. In *Arabidopsis*, resistance to specific strains of *P. syringae* is controlled by a single gene designated RPM1. In soybean, resistance to specific *P. syringae* strains is controlled by the gene Rpg1. There are at least 4 different versions of Rpg1, each of which is specific to a different strain of *P. syringae*. RPM1 has already been isolated. We are now concentrating on isolating Rpg1. By comparing the amino acid sequences of RPM1 and the four different versions of RPG1 we will gain insight into which specific amino acid sequences are important for conferring specificity. This in turn should help us design plant disease resistance genes with new and broader specificities.

9901487 Rice Deletion Mutants for Dissection of Quantitative Resistance

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Grant 99-35300-7682; $300,000; 3 Years

As we seek less dependence on chemicals for crop protection, achieving durable or long-lasting disease resistance is one of the most important objectives in disease resistance research. In rice, durable resistance is attributed to both single, dominant resistance genes (qualitative resistance) and to the collective contribution of many genes (quantitative resistance). The exact biochemical mecanisms of such resistance and genetic interactions among the genes are far from clear. The successful cloning of many single, dominant resistance genes has led to a better understanding of the molecular biology of the steps for induction of qualitative resistance, yet much remains to be discovered about the genes that collectively confer quantitative resistance, i.e., the defense response (DR) genes. Our overall goal is to understand quantitative resistance in rice. To approach this goal, we will develop a collection of rice mutants with deletions in DR genes. Two essential resources for implementation of this project have been produced: 1) a collection of candidate DR genes, and 2) a large collection of rice deletion mutants (approx. 40,000) for forward and reverse genetics. Using these two resources, we propose to develop an efficient screen to detect mutants with deletions in specific DR genes. DNA from 10,000 deletion lines will be arrayed on membranes and screened by gene specific hybridization probes and PCR methods to identify genomic changes (deletion mutations) in DR genes. Rice with deletions in DR genes will be inoculated with the blast fungus, *Magnaporthe grisea* and the bacterial blight pathogen, *Xanthomonas oryzae pv. oryzae* to determine the effects of the mutations on disease resistance and susceptibility. Knowledge of the effects of individual DR genes will provide the basis to pyramiding genes for quantitative resistance in rice. Successful application of the mutant screen will have broad utility in identifying mutants deleted in candidate genes involved in other metabolic pathways.

9901395 Maize Resistance Genes Expressed in Transgenic Cereals

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Grant 99-35300-7702; $120,000; 2 Years

The long-term goal of the proposed research is to understand the mechanisms involved in the broad-spectrum resistance of the genes in the maize Rp1 locus toward *Puccina sorghi* and to determine whether Rp1 genes can function in other cereals such as wheat. This proposed research is expected to demonstrate that: 1) over-expressed Rp1-D gene in maize is responsible for resistance against maize leaf rust; and 2) Rp1-D has a similar result against wheat leaf rust when the gene is introduced into wheat. A full-length genomic sequence of the recently cloned Rp1-D gene of maize, as well as the over-expressed coding region, will be introduced into maize and wheat via particle bombardment. Gene integration, stability, expression and inheritance will be assessed by southern-, northern-, and western-blot hybridization analyses. Based

on Rp1-D expression levels, five to ten transgenic maize and wheat lines will be selected for pathogen inoculations. Transgenic maize plants will be challenged with *P. sorghi* (maize leaf rust) and *P. polysora* (southern leaf rust). The transgenic wheat will be challenged with pathotypes of *P. recondita*, (wheat leaf rust). Plants will be scored based on the number and the size of rust pustules as well as a delay in pustule formation. This proposed research should answer fundamental questions pertaining to the scope of the RP1 resistance genes. If successful this research will provide a new source of resistance in wheat against rust not obtainable by conventional breeding techniques.

9901406 Informatics-based Comparative Mapping
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Grant 99-35300-7901; $60,000; 1 Year

The Human Genome Initiative will eventually reveal the genetic code for all of the genes and intergenic DNA in the human genome. For domesticated plant species, no similar investment will be made. Instead, the strategy will be to sequence the small genomes of model plant species such as rice and *Arabidopsis*, and to use this information for directed sequencing of "islands" of important genes in economically important crop species. Identification of gene islands in existing maps for model species is effective for direct gene search in related species because in these cases, gene order is conserved in large blocks. This project will develop software tools to facilitate such comparisons between gene maps of rice and maize. If such tools can be built for this pair of species, then the software tools should be adaptable to any arbitrary pair of plant species.

9901417 Genomic Diversity of Sequences Homologous to Disease Resistance Genes in *Populus*
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Grant 99-35300-7738; $200,000; 2 Years

Diseases are major impediments to the sustained success of poplar plantations and that of many other intensively grown tree species, limiting their viability, productivity, and adoption. New diseases have often devastated plantations, and rendered highly productive tree clones obsolete. Recent fundamental insights into resistance gene structure from molecular studies offer new avenues for reducing the incidence of disease in tree plantations via biotechnology.

Based on the similarities among resistance genes at the DNA level that have been found in diverse plant species, we will identify a large number of new potential resistance genes from black cottonwood (*Populus trichocarpa*). We expect to identify DNA sequences of several hundred potential resistance genes, analyze their evolutionary similarities, map the positions in the genome of two-dozen representatives, and study their association with disease resistance loci that we will map via conventional genetic means. In the medium term, the gene sequences we develop should provide the basis of new, highly automated systems for the identification of strongly and stably resistant trees, and allow the isolation and asexual transfer of complete resistance genes via genetic engineering. In addition to their potential applications via biotechnology, the new gene sequences will enable new fundamental studies of resistance gene diversity, evolution, and ecological adaptation in wild tree populations.

9901294 Establishment of a Genetically and Physically Anchored EST Resource for Barley Genomics
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Grant 99-33500-7694; $600,000; 3 Years

The discovery, characterization, and exploitation of agriculturally important genes is critical to meet the food security needs of the next century and for fundamental understanding of plant biology. Traditionally, gene discovery programs followed a "one-gene-at-a-time" approach, which is costly and time consuming. In the age of genomics, scientists are taking global approaches to gene discovery such as, sequencing all of the transcribed genes of an organism in the form of expressed sequence tags (ESTs) to determining the complete DNA sequence of an organism. Which genomics strategy to choose typically depends on the size of the genome and the funding available. For the small-genome model plants, *Arabidopsis* (120 Mb) and rice (430 Mb), the scientific community has supported several EST projects and the complete genomic sequencing of both genomes. In contrast, complete sequencing of large-genome plants is not yet practical, especially for crops with genome sizes well beyond the size of the human genome of 3,000 Mb, such as barley (5,000 Mb). A more affordable solution to gene discovery in such crop plants is to develop EST databases which will provide a wealth of information in a relatively short time. The long-term objective of this proposal is to establish and provide a well-characterized barley EST resource for the genomics community. The specific objectives of this proposal are to develop high quality cDNA libraries from important developmental stages and tissue types, sequence, characterize and display 50,000 barley ESTs, and anchor prioritized ESTs to the barley and rice BAC libraries.

9901420 IUFRO Conference: "Future of Pine Genomics"

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Grant 99-35300-7846; $5,000; 1 Year

"The Future of Pine Genomics" is the topic of the 1999 International Union of Forest Research Organizations (IUFRO) Conference for Working Party 2.02.20 Southern Pine Genetic Resources. The conference will be held from July 11 to July 13, 1999 at the Fairmont Hotel, New Orleans. The conference will precede the Southern Forest Tree Improvement Conference for breeders and forest geneticists.

This IUFRO Conference topic is based on the need for interdisciplinary dialogue within the pine genomics and breeding community. Many pine community concerns were identified in a 1998 listening project jointly sponsored by Texas A&M University and the North Carolina Biotechnology Center. How do we access germplasm in the absence of public breeding programs? How shall we share research tools and germplasm in the absence of a national stock center or a germplasm repository? How do we access DNA sequence databases in a timely manner? Unlike other commodity groups, the loblolly pine genetics community has unique problems. There are no stock centers and no public sources of germplasm. All research tools and germplasm access represents negotiated arrangement which tends to preclude researchers outside forestry from using these resources. Given the importance of comparative genomics, this is a potential hindrance to the larger plant genome community.

The conference will address these timely questions. Strengthening interdisciplinary links as well as raising awareness will initiate discussion of community issues. Our goal is to propose a small germplasm/stock center for shared research germplasm and genetic tools. This proposal requests travel funds for internationally- and nationally-known invited speakers outside forestry.

9901325 Molecular Genetics of the Barley Stem Rust Resistance Gene Families

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Grant 99-35300-7876; $300,000; 3 Years

The main objectives of this grant are (1) to clone and characterize the genes that code for barley stem rust resistance and (2) to understand the genome organization differences and similarities between barley and rice in the stem rust resistance gene regions. Stem rust is a serious barley disease in some US barley growing regions. It has been effectively controlled by genetic resistance incorporated into the best adapted cultivars. The genes that encode this resistance have been effective and durable. However, they can not be expected to maintain a high level of resistance forever and will eventually break down like most other disease resistance genes. Therefore, it is important to understand how and why they are effective in order to facilitate identification or construction of new genes with comparable effectiveness. The studies comparing genome organization of rice with barley are important because barley has a very large genome and the related cereal rice genome is 11X smaller. Thus, we and others, would like to exploit rice to the maximum extent possible to facilitate map-based cloning of agriculturally important genes from barley. With the rice genome sequencing in progress, it is important to understand how the rice genome is related to barley in order to use the rice information most effectively for cloning barley genes.

9901304 Molecular Genetic Analysis of Vernalization in Crucifers

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Grant 99-35300-7724; $183,151; 3 Years

Many plant species have evolved systems to regulate the timing of flowering in response to environmental cues such as changes in daylength and temperature. The goal of this proposal is to further our understanding of the mechanisms that regulate flowering in response to cold exposure. The promotion of flowering resulting from cold exposure is known as vernalization. Biennials are a class of plants that have an absolute requirement for vernalization. The biennial habit is an important feature of many crop plants. For example, crops such as cabbage, carrots, beets and onions do not generally flower during the first growing season. If they did flower the crop would be rendered useless; i.e., the presence or incorporation of the biennial habit into such crops was an essential component of their domestication. We propose to identify and characterize a gene from *Arabidopsis thaliana* that is responsible for preventing flowering until vernalization is complete. We will then study the role of homologous genes in the cabbage family (Brassicas) to understand how biennialism is regulated in this crop. The molecular understanding of the mechanisms that regulate flowering could be of great importance in agriculture because most crops are either the product of flowering (i.e., seeds and fruits) or the vegetative parts of the plant (such as leafy vegetables or biomass crops). Manipulation of flowering could allow adjustment of the timing of seed set or fruit development to increase yields. Delaying or preventing flowering of crops in which the vegetative parts are used for food or biomass is likely to increase yield because energy would not be diverted to reproduction.

PLANT GENETIC MECHANISMS

Panel Manager - Dr. Robert Martienssen, Cold Spring Harbor Laboratory
Program Director - Dr. Liang-Shiou Lin

This program area supports studies addressing the basic cellular, molecular and genetic processes which contribute new information required for the development of novel approaches to crop and forest improvement. Innovative research is emphasized in the following areas: (1) characterization of agriculturally important genes and gene products, (2) relationship between gene structure and function, (3) regulatory mechanisms of gene expression, (4) nucleic acid metabolism and protein-nucleic acid interactions, (5) interactions between nuclear and organellar genomes, (6) mechanisms of recombination, transposition, replication, and repair (7) genetic mechanisms affecting diversity in natural and crop/forest plant populations.

9901366 Tissue Specific Regulation of the Maize Anthocyanin Pathway
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Grant 99-35301-7791; $200,000; 3 Years

Our research seeks to understand how genes are turned on and off in different tissues of the plant. We study two genes, *b* and *pac1,* that regulate the transcription of the anthocyanin biosynthetic genes in a tissue-specific manner. The *b* gene, which has many alleles with distinct tissue and developmental specific expression patterns, encodes a transcription factor that works together with a MYB-like protein encoded by the *c1* or *pl* genes. Our previous work identified the sequences necessary for the aleurone-specific expression of the *B-Peru* allele. We have also identified 32 novel mutants that reduce the aleurone pigmentation directed by *B-Peru* and *C1*. We have shown that one of these mutants, *pac1, is* a new, tissue-specific regulator required for anthocyanin biosynthetic gene expression that acts independently of the other known regulators. We will clone and characterize *the pac1* gene, which should lead to a better understanding of how the anthocyanin regulatory system works. Of the 14 new mutants analyzed, two appear to reduce the expression of *B-Peru.* We will characterize the remaining 18 mutants and focus our attention on those that effect the expression of the regulatory genes. All of these mutations are pleiotropic, affecting seed viability as well as anthocyanin pigment. As these genes may affect other processes in addition to anthocyanin biosynthesis, further characterization and cloning of genes defined by these mutants may lead to a better understanding of the regulators involved in the initiation and development of the aleurone layer. A better understanding of the processes and requirements of tissue-specific expression of a biochemical pathway will greatly enhance the potential of genetic engineering in crop plants by enabling the reliable expression of multi gene pathways in the tissue of choice. The regulatory pathways and mechanisms identified in our studies should also be useful in understanding and engineering tissue-specific gene expression in other important agronomic plants.

9901389 Brassinosteroid Signal Transduction
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Grant 99-35301-7903; $240,000; 3 Years

This project describes experiments designed to understand how plant cells respond to the steroid hormone, brassinolide (BL). Recent studies have indicated that both the biosynthetic and response pathways of BL can be manipulated to affect plant stature. During the previous funding period, we identified several genes in the BL biosynthetic and signaling pathways, including the putative receptor. We now propose experiments to manipulate BL levels using transgenic plants that overexpress either a BL biosynthetic enzyme or an inactivating enzyme. We also want to analyze in more detail the molecular mechanisms of BL perception and response. Using knowledge gained from the cloned BL biosynthetic and receptor genes, it may be possible to create transgenic plants with increased growth and yield and enhanced disease resistance. Modifications of the receptor could also lead to plant varieties that are more responsive to BLs or less susceptible to disease. These transgenic plant varieties would circumvent the need for expensive chemical syntheses, as well as the requirement for manual applications of the hormone. Thus, research on plant steroid hormones might lead to changes in agriculture that would be both economically and environmentally beneficial.

9901502 Induction of Flowers in Transgenic Plants
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Grant 99-35301-8047; $280,150; 3 Years

Most plants use day length (which changes with latitude) to control when they start to form flowers, and many crop plants are therefore limited in the geographic area where they can be grown. Furthermore, breeding programs aimed at introducing new traits into crop plants are often limited by long flowering times. Our long-term objective is to understand

the genetic and molecular basis of flower initiation to such an extent that one can manipulate this process at will. The immediate objective of this proposal is to understand how two related genes, *FT* and *TFL1*, affect flowering of the model plant *Arabidopsis* in opposite ways. By modulating the relative levels of *FT* and *TFL1*, it is already possible to fine tune flowering time of *Arabidopsis* over a wide range. However, the molecular mode of action of *FT* and *TFL1* is not known, although their properties suggest that they are involved in the relay of a floral inductive signal. The proposed work will clarify how, when and where *FT* and *TFL1* act to control flowering. Being able to manipulate flowering in a rational way would have important implications for the long-range improvement in and sustainability of U.S. agriculture and forestry. For example, many crops cannot be grown in certain areas, because they will not flower at an appropriate time. Conversely, flowering can be undesirable, as in many forage crops. Limitations imposed either by insufficient or inappropriate flowering could be overcome through the production of transgenic plants with altered control of flower initiation.

9901489 Genetic Components Required for Paramutation at the Maize *pl* Locus

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Grant 99-35301-7753; $120,000; 2 Years

The genesis, maintenance, and manipulation of genetic variation are of primary agronomic importance, yet the very nature of genetic variation is poorly understood. This work addresses an example whereby such variation is exhibited by a single gene. The *pl (purple plant)* gene in corn controls the production of purple pigment. One allele (a particular form) of the *pl* gene can exhibit a range of activity conferring weak to intense coloration. Particular levels of activity are heritably changed through interactions with the other *pl* allele; this influence of one allele on another is called paramutation. Thus, using certain combinations of *pl* alleles, plant color can be either enhanced or reduced. This is reminiscent of the processes of inbreeding depression and heterosis (hybrid vigor); it is expected that these studies on an experimentally amenable pigment gene will lend insights into the mechanistic basis of both processes. The long term goal of this work is to elucidate the molecular basis of these allelic interactions that occur during paramutation; what DNA sequences are required and what cellular proteins mediate the process. As a first step, several genes required to mediate *pl* paramutation have been identified by mutational analysis. The primary aim of this project is to further characterize these mutations and identify the cellular proteins encoded by these genes. Results of this study will identify molecular models to be subsequently tested. A thorough understanding of this process should eventually lead to novel approaches for both traditional and marker-assisted breeding strategies.

9901390 Molecular Mechanism of *EMF* Regulated Shoot Development in *Arabidopsis*

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Grant 99-35301-7984; $210,000; 3 Years

Our long-term goal is to determine the genetic mechanism controlling shoot development and flowering in plants and to manipulate the mechanism for the improvement of agronomic and forest species. Plant development is characterized by phase changes marked by the successive appearance of morphologically distinct organs. Shoot maturation from embryo, to juvenile, to adult and finally to reproductive phases can be recognized by the sequential appearance of lateral organs such as cotyledons and leaves, and the floral organs-sepals, petals, stamens, and carpels. Maturation state or plant age determines the temporal and spatial pattern of development and the morphology of the organs produced The *EMBRYONIC FLOWER (EMF)* genes may encode factors that regulate shoot maturation. They are essential for the specification of the vegetative phase in *Arabidopsis thaliana*, a genetic model plant, and also influence the reproductive phases of shoot development. EMF2 is a zinc finger transcription factor, while EMF1 is a novel protein, possibly a transcriptional regulator involved in a signal transduction pathway. Our specific objectives in this proposal are to confirm and characterize the molecular function of EMF1, to test the hypothesis that EMF1 and EMF2 activities repress reproductive development in *Arabidopsis*, and to study how hormones, day length, and the genes involved in flowering time and flower development regulate EMF1 and EMF2 activities. Once the function and regulation of *EMF* genes are proved, we may use *EMF* genes to manipulate the flowering time of crops, e.g., we may introduce sense *EMF* construct into crops to postpone the transition from vegetative to reproductive growth to generate more leaves for leafy crops, and antisense *EMF* to accelerate this transition to speed up the production of seeds and fruits.

9901339 Evolution of Genepools and Interploidy Gene Flow in Shaping Wheat Diversity

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Grant 99-35301-7905; $200,000, 3 Years

The goal of this study is to characterize genetic variation and its sources in wheat (genus *Triticum* L.) and use it in deciphering the evolution of wheat. The proposed research will focus on the two most important species of wheat, the tetraploid *Triticum turgidum* (macaroni wheat) and the hexaploid *Triticum aestivum* L. (bread wheat). Both species are

important for the economy of this country. Genetic evidence shows that *Triticum aestivum* evolved by hybridization of tetraploid wheat *T. turgidum* with goatgrass *Aegilops tauschii*. Archaeobotanical evidence suggests that this event occurred some 8,000 years ago, probably under cultivation. Genetic diversity within and relationships among the genepools of all forms of *T. turgidum* and *T. aestivum* will be estimated and used in the reconstruction of events that led to the evolution of the modern forms of *T. turgidum* and *T. aestivam.* Additionally, genetic relationships and distribution of diagnostic alleles among the various forms of tetraploid and hexaploid wheats will be used to assess the role which tetraploid *T. turgidum* played in shaping the genetic and morphological diversity in *T. aestivum* and in the origin of primitive forms of *T. aestivam*.

9901578 Identification of the Molecular Determinants Responsible for Resistance to Geminivirus Infection in *Phaseolus vulgaris*

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Grant 99-35301-7902; $130,000; 2 Years

In the United States, plant-virus-induced diseases are responsible for significant losses to crops such as tomatoes, peppers, melons, and beans. Virus-resistant plants are the most desirable method to control plant viruses. Geminiviruses are single-stranded DNA viruses transmitted by insects (e.g. whiteflies). These viruses are responsible for significant vegetable crop losses, and a very devastating geminivirus of tomato (tomato yellow leaf curl virus) was recently introduced into Florida. Unfortunately, most crops possess no natural resistance to geminivirus infection. Using a bean dwarf mosaic virus (BDMV)-green fluorescent protein (GFP) reporter system, we have demonstrated that a resistance response in the bean cultivar Othello involves a blockage of long-distance spread of the virus, and is associated with a hypersensitive response (HR). In contrast, the related bean golden mosaic virus (BGMV) infects and spreads, long-distance, in cv. Othello. We will utilize these two viruses, one of which elicits the resistance and one of which does not, to identify the viral gene product(s) that triggers the resistance response. This will be accomplished by generating a series of hybrid viruses and testing their capacity to elicit the resistance response. Once the elicitor is identified, it will be inserted into BGMV to determine if cv. Othello will be now be resistant to BGMV. This information will provide insight into the intercellular location of the resistance response, and will initiate a dissection of the virus-host interaction involved in the resistance response. The long-term goal is to clone the geminivirus resistance gene and use it to make geminivirus-resistant crop plants.

9901347 Genetic Diversity and Evolution of a Defense Trait in Maize and Its Wild Relatives

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Postdoctoral Fellowship; Grant 99-35301-8076; $90,000; 2 Years

Crop loss to herbivores and pathogens is a major limitation to agricultural production. Herbivores and pathogens are also a major selective force in natural ecosystems. Although the mechanisms plants have evolved to limit tissue and seed loss to herbivores (defense mechanisms) have been subject to intensive investigation by biochemists, ecologists and evolutionary biologists, relatively little is known about the evolutionary dynamics of defense mechanisms. The main goal of the proposed research is to analyze information contained in DNA sequences to understand evolutionary dynamics of one type of plant defense, protease inhibitors, in maize and its wild relatives. The specific objectives are to determine i) whether defense mechanisms are changing continuously or are maintained over long periods of time, and ii) the temporal and geographical scales at which defense mechanisms evolve. These objectives will be met by isolating and sequencing protease inhibitor genes from 10-20 plants from each of two geographically distinct populations of maize and two closely related wild species. Recently developed statistical techniques will be used to analyze the information contained in the gene sequences. In addition to providing unique insights into the evolutionary dynamics of plant defense this research will i) identify genetic resources that may be useful in crop improvement, and ii) may provide information that is useful in developing efficient strategies for sampling genetic diversity for defense mechanisms from natural populations of agronomically important plant species.

9901375 Analysis of the Protein-Protein Interactions Required for Efficient Translation

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Grant 99-35301-7866; $210,000; 3 Years

The production of protein is essential to all living organisms, including plants. Like other species, plants regulate protein synthesis through global and specific mechanisms. Virtually all plant mRNAs contain a cap structure at the 5'-terminus and a poly(A) tail at the 3'-terminus. Efficient translation in plants requires a synergistic interaction between the 5' cap structure and the poly(A) tail of an mRNA. This interaction is mediated through a physical interaction between the poly(A)-binding protein (PABP) that binds to the poly(A) tail and the cap-associated initiation factors, e.g., eIF4G, eIFiso4G, and eIF4B. The consequences of the interaction between PABP and these factors is to increase their binding to an mRNA. An analysis of the interaction between PABP and the cap-associated initiation factors at the molecular level will be

carried out as the next step in understanding how this critical interaction is regulated during protein synthesis. Specifically, we will: Identify each domain within PABP that is required for interaction with eIF4G, eIFiso4G, and eIF4B, and secondly: Identify the domain within eIF4G, eIFiso4G, and eIF4B that is required for interaction with PABP. These studies will be the first to examine the interactions between the proteins bound to the termini of mRNAs in order to better understand how they interact and consequently function to achieve the global control of protein synthesis in plants.

9901358 Intralocus and Intragene Recombination in the *Gli-B1* and *Glu-B3* Loci in Wheat

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Strengthening Award; Grant 99-35301-7593; $120,000; 2 Years

We will study the pattern of recombination in the multigene loci *Gli-B1* and *Glu-B3* in wheat, encoding gliadins and low molecular weight (LMW) glutenins. These two storage proteins contribute over one half of the observable variation in the technological properties of wheat. Because of very tight linkage between the two, little is known about the effects of allelic variation at each locus, their organization, or the contribution of individual genes to the properties of wheat. We will saturate the critical region of the chromosome arm lBS with at least 800 cross-overs per 1 cM produced at two levels of chromosome pairing stringency control (normal, *Phl,* and mutant, *phlb)* by using a recombination-enhancing translocation and screening for flanking markers. This should let us determine the number and the order of genes present at each locus, to break linkages between the two loci and between individual genes in each locus, and to generate new hybrid alleles. We will develop PCR methods for high resolution mapping within the loci and to produce DNA markers for individual alleles. We expect that the materials and methods developed in this study will let us define in the future the criteria of the DNA sequence similarity for crossing over as well as generate convenient DNA markers for various alleles of storage proteins for plant breeding. This study will also generate genetic stocks of wheat for field tests of the technological properties of new genetic variation.

9901488 Plant Responses to Phytophagous Insects: The Silverleaf Whitefly-Squash Interaction

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Strengthening Award; Grant 99-35301-8077; $100,000; 2 Years

The silverleaf whitefly is a severe agronomic pest and causes the developmental disorder squash leaf silvering. Squash genes *(SLW)* induced by the silverleaf whitefly have been identified and will increase our understanding of plant-defense responses to phloem-feeding insects. Additionally, if *SLW* genes are strictly correlated with the leaf slivering disorder, this research will lead to an understanding of the mechanisms that cause leaf silvering. Long-range goals are to design of cogent transgenic strategies to ameliorate the developmental disorders induced by the silverleaf whitefly and to identify the signals that induce *SLW* expression. *SLW1* encodes a M20B peptidase that is expressed in flowers and fruit and in response to whitefly feeding, jasmonic acid, ethylene, and water deficit. *SLW3* encodes a leaf-specific -glucosidase that is only induced by whitefly feeding and water deficit. *SLW3 is* regulated by a novel signaling pathway, since it does not respond to known defense/wound signals. We will determine if the levels of SLW1 and SLW3 proteins correlates with their RNA levels. Since access to substrates for these enzymes will be determined by their localization in the cell, the location of the SLW proteins will be determined. The levels of *SLW* RNAs and proteins will also be assessed in (a) silvering lines of *Cucurbita,* pea, and tomato and silvering-resistant *Cucurbita,* (b) plants that exhibit other silverleaf whitefly-induced developmental disorders, (c) *Arabidopsis* during whitefly feeding, water deficit and development. Finally, to determine the likely role of the *SLW1* M20B peptidase and the *SLW3* - glucosidase their substrates will be determined.

9901351 The Role of PBF in Seed Storage Protein Gene Expression

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Grant 99-35301-7906; $120,000; 2 Years

We have recently isolated an endosperm-specific cDNA encoding a DNA binding protein that interacts with the highly conserved Prolamin Box. The Prolamin Box is a *cis*-acting element that is conserved in location and sequence within the promoters of a large number of cereal storage protein genes. The protein that binds this sequence motif, the Prolamin Box binding Protein (PBF), is a member of a recently identified and poorly characterized class of plant Cys2-Cys2 type zinc finger proteins (DOF proteins). Our research end that of others implicates PBF as an important regulator of seed storage protein gene expression.

To understand the role of PBF in the process of seed storage protein gene expression we will identify mutant alleles through reverse genetics and/or through single seed EMSA screens of *dek* mutants linked to PBF. In addition, we will generate transgenic maize lines that over-express PBF to test specific predictions of its function, and to determine what effect elevated levels of PBF will have on zein protein accumulation and endosperm development.

Cereal seed storage proteins are one of the primary sources of proteins in the diets of humans and their agricultural animals worldwide. The bulk of these proteins are produced solely in the endosperm, a highly specialized tissue devoted to starch and protein biosynthesis and storage. The possibility of designing effective strategies to genetically engineer seed crops with improved nutritional value and/or with the capacity to produce peptides having pharmaceutical or medical importance can be greatly improved through an increased understanding of the mechanisms regulating seed storage protein gene expression.

9901579 *Cis*- and *Trans*-Modifiers of Meiotic Recombination

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Grant 99-35301-8515; $150,000; 2 Years

Meiotic recombination is a central mechanism by which genetic diversity is generated. Such genetic diversity is fundamental to evolution and to many of the strategies used to improve crop species. Considerable evidence indicates that meiotic recombination does not occur evenly throughout plant genomes; rather, recombination "hot" spots and "cold" spots exist. Most fine-structure analyses of recombination in plants have focused on genes and have therefore not assayed recombination rates specifically in non-genic regions. The 140-kb, multi-genic *a1-sh2* interval from maize is being used as a model to determine the relative contributions of genic and non-genic recombination. Because this approach identifies recombination hot spots without making prior assumptions regarding their molecular nature, it will begin to test two hypotheses formulated to explain the fact that large regions of the genome experience very different rates of recombination. In addition, this research will utilize unique genetic stocks to separate the genetic effects of *cis* or *trans* modifiers of recombination. Stocks that carry *cis*-acting modifiers that modulate rates of recombination across the *a1-sh2* interval, will be used to test the hypothesis that such modifiers can also affect the distribution of recombination breakpoints. Similarly, stocks that carry unique configurations of the *a1-sh2* interval will be used to characterize the effects of specific *cis*-acting factors on recombination rates. Finally, another set of genetic stocks that carry *trans*-acting modifiers that modulate rates of recombination across the *a1-sh2* interval, will be used to test the hypothesis that *trans*-acting modifiers can affect the distribution of recombination breakpoints.

9901384 Defining the Roles of Interactions Between the Plant Nuclear Poly(A) Polymerase and Other Factors

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Grant 99-35301-7904; $130,000; 2 Years

The proposed study examines a crucial aspect of gene expression in higher plants, namely the addition of poly(A) tracts to messenger RNAs. A clear understanding of mRNA polyadenylation is important, for a complete appreciation of the growth and development of plants, and for efficient, rapid application of genetic engineering technology to crop improvement. In this study, the genes that encode nuclear poly(A) polymerases in plants will be isolated and characterized. In addition, the interactions of this enzyme with other factors will be studied. These experiments will help to clarify the differences that exist between plants and other organisms with respect to mRNA polyadenylation. They will also explore a new model for the function of the nuclear poly(A) polymerase, one that involves nuclear RNA turnover as well as polyadenylation. With their completion, these studies will contribute towards the design of improved means for expressing genes in plants, and for the use of RNA-based technology (sense, antisense) to develop improved agricultural products.

9901509 Genetic Analysis of Endosperm Factors in Maize

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Grant 99-35301-7621; $180,000; 3 Years

The endosperm of the grains, which contains reserves of starch and protein for use by the plant seedling, is an important component of the world's food supply. Experiments are proposed to study factors that affect the size and development of the endosperm in maize. When these Endosperm factors (Efs) are missing from the paternal gamete, the endosperm is smaller. This phenotype can not be rescued by introducing extra copies of the same chromosomal region through the female parent and in fact this situation causes a maternal enhancement making the endosperm even smaller. The cumulative action of the responsible genes has been postulated to be responsible for the endosperm failure that occurs in crosses between plants differing in the number of genome copies. The maternal enhancement factors in the genome will be localized along the length of the chromosome arms where they reside. This information will be used to test the hypothesis that the maternal enhancement and the male Ef regions are coincident. Lastly, the four strongest Ef regions in the genome will be combined and varied together to test the hypothesis that the endosperm failure that occurs in crosses between plants with different genome copy numbers is due to the cumulative action of the Efs. Determination of the unusual genetic interactions that are involved with the endosperm will be useful for standard breeding programs and biotechnological approaches to improving yield and protein quality among the grains.

9901501 Plant Polypyrimidine Binding Proteins as Chromatin Derepressors

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New Investigator Award/Strengthening Award; Grant 99-35301-8023; $118,350; 2 Years

Plant and animal cells produce a form of histone H3 protein that assists the maintenance of chromatin structure in differentiated cells. Genes for such histone H3 variants in plants are constitutively expressed despite the presence of typical cell cycle control promoter elements. Phylogenetic gene analysis suggests that polypyrimidine (PPY) sequence elements are required for the constitutive expression. They are located among the promoter elements, in the untranslated regions (UTRs) and in the introns. We hypothesize that they bind proteins similar to the *Drosophila* GAGA factors, which cause chromatin derepression by preventing nucleosome assembly. In *Arabidopsis*, intron insertion with these elements caused strong increase in reporter gene expression and loss of the cell cycle control of a histone promoter. An alfalfa histone H3 promoter, which contains no intron in the 5' UTR but instead similar PPY motifs, conferred high, constitutive reporter gene expression . Gel shift analyses detected specific interactions between plant nuclear proteins and PPY motifs in the 5' untranslated and intragenic regions of the alfalfa gene. This project has the specific aim to confirm whether PPY elements cause high, constitutive gene expression in transgenic plants. Secondly, PPY-binding proteins will be purified from plants, sequenced and related cDNA clones will be isolated. Transgene silencing by heterochromatin is a major problem in plant biotechnology. The use of PPY sequences as natural gene de-repressors in the promoters and other parts of plant genes will be an invaluable tool to prevent gene inactivation and to maintain high expression of a desired gene throughout plant generations.

9901364 Chloroplast DNA Methylation and Inheritance in *Chlamydomonas*

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Grant 99-35301-7765; $190,000; 3 Years

Plant chloroplasts contain their own genomes that are inherited by a different mechanism than the nuclear genome: one parent contributes most or all of the chloroplast DNA (cpDNA) received by progeny generating an inheritance pattern that is termed uniparental. Uniparental inheritance is not always the result of the differential cytoplasmic contributions of small male pollen versus large female eggs; some plants display paternal inheritance despite the numerical disadvantage faced by male chloroplast DNA in zygotes. Since chloroplast genes may be targets for genetic engineering of plants, it is important to understand the mechanisms governing their inheritance. The unicellular alga *Chlamydomonas reinhardtii is* an excellent model system for understanding the genetic and molecular bases of uniparental chloroplast inheritance. *C. reinhardtii* exists as two mating types, mt^+ and mt^- that can fuse to form a diploid zygote. Within hours of zygote formation, the cpDNA contributed by the mt^- parent is destroyed, leading to uniparental inheritance of cpDNA from the mt^+ parent. We are investigating the role of methylation, a covalent cpDNA modification that occurs during gametic differentiation. mt^+ cpDNA becomes methylated in gametes to a much greater extent than mt^- cpDNA, and drugs that inhibit mt^+ cpDNA methylation increase the transmission of mt^- cpDNA to progeny. We propose to determine which cpDNA sequences are methylated in gametes and zygotes, and will use molecularly "tagged" cpDNA genomes to monitor the fate of zygotic mt^+ and mt^- cpDNA when methylation has been blocked. We will also use cloned *C. reinhardtii* methyltransferase genes to ask whether ectopic methylation can enhance transmission of mt^- cpDNA.

9901577 *In Vitro* Approaches to Studying RNA Polymerase I Regulation in Plants

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Grant 99-35301-7865; $240,000; 3 Years

Proteins in all living cells are made from building blocks called amino acids by cellular machines known as ribosomes. Ribosomes are complicated chemical machines with many parts, including four different RNA molecules and approximately 85 proteins. The three largest ribosomal RNAs are synthesized (transcribed) by a multi-protein enzyme known as RNA polymerase I. Initially, one long RNA is transcribed by polymerase I. This RNA transcript is subsequently cut into three pieces by other enzymes. The rate at which ribosomal RNAs are produced appears to dictate the rate at which complete ribosomes are assembled. The rate of ribosome production, in turn, controls the capacity of the cell to make proteins which, in turn, controls the rate at which cells can grow and divide. Thus an understanding of how RNA polymerase I is regulated is expected to directly contribute to our understanding of growth regulation. As a first step towards this understanding, we have developed a cell-free system from broccoli which can carry out RNA polymerase I transcription of ribosomal RNA in the test tube. Extensive purification of the transcription activity suggests that it may be one large complex, possibly containing dozens of proteins. An alternative is that multiple individual proteins or protein sub-complexes co-purify but are not physically associated within a single, RNA-synthesizing machine. The major goal for the funding period is to distinguish between these possibilities and determine the number of proteins likely to play a role in rRNA transcription in plant cells.

9901742 Genetics and Biochemistry of Wheat Grain Hardness Genes

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New Investigator Award/Strengthening Award; Grant 99-35301-7764; $120,000; 2 Years

Grain hardness in wheat is a primary factor of wheat flour quality. Grain texture or hardness is a function of the physical texture of the endosperm, and influences such quality parameters as milling energy, particle size, starch damage, baking quality, and water absorption. These quality parameters in turn, strongly impact the commercial usage and value of wheat. Hard wheat flour is used primarily for bread, while soft flour is used primarily in cookies, cakes, and pastries. A single locus, *Hardness (Ha)*, controls the difference between hard and soft wheat. This gene difference is marked by soft *(Ha)* or hard (*ha*) textured endosperm and large (*Ha*) or small (*ha*) amounts of the marker protein friabilin on the surface of starch granules. Sequence or transcriptional alterations in the two genes that encode friabilin, *puroindoline a* and *b (pinA* and *pinB*, respectively), are inseparably linked to the wheat *Hardness* locus in wheat that is hard textured. The unbroken linkage between putative mutations in these two genes and hard textured endosperm suggests that they together comprise the *Hardness* gene. Additionally, the putative mutation in *pinB*, a simple glycine to serine change is common in hard wheats that are softer than those with the *pinA* alteration, a null mutation. The data suggest that the *puroindoline* genes act together, to effect softness. The experiments proposed are directed at current shortcomings in our knowledge of endosperm grain texture and the project aims to conclusively identify the genes controlling endosperm texture in wheat. Specifically, the objectives of this project are to conclusively identify the *pinA* and *pinB* genes as functional components of the *hardness* gene using transgenic plant technology. The second objective will quantify flour quality difference between hard wheats containing an alteration in *pinB* versus those devoid of the PINA protein.

9901383 Genetic Control of Downy Mildew Resistance in *Arabidopsis*

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Grant 99-35301-7848; $210,000; 3 Years

We study the molecular basis of disease resistance in plants. Loss of plant harvest yield due to pathogens and continued application of environmentally unsound pesticides are major agronomic problems. It is hoped that direct isolation and manipulation of single genes conferring pathogen resistance will help alleviate these problems. We have chosen, in our USDA funded research, to analyze the infection of a model plant, *Arabidopsis*, with the parasite which causes downy mildew disease. In the previous funding period we isolated a so-called disease resistance gene termed *RPP8*. This gene makes plants resistant to a particular strain of pathogen which causes downy mildew disease. We found that both *RPP8* and another disease resistance gene termed *RPP7* are regulated differently than other disease resistance genes analyzed to date. Our current Specific Aims are to use genetics and molecular biology approaches to identify and isolate these novel regulators.

9901355 Molecular Basis of Recognition Specificity in Plant Disease Resistance

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Grant 99-35301-7973; $240,000; 3 Years

Diseases of plants result in great economic losses and yet relatively little is known about how plants naturally recognize and defend themselves against disease-causing organisms. To increase our understanding of recognition and defense responses in plants we study resistance in tomato to speck disease caused by the bacterium *Pseudomonas syringae* pv. tomato. Tomato plants that contain the *Pto* disease resistance gene are able to specifically recognize bacteria that contain the gene *avrPto*. Previous research showed that the basis of this specific recognition is the physical interaction between the Pto and the AvrPto proteins. With USDA support (NRICGP grant no. 96-35301-3148) we found that certain amino acids in the *Pto* protein, including a threonine residue at position 204, determine *Pto* recognition specificity for the AvrPto protein. Because *Pto* encodes a serine/threonine protein kinase it is possible that phosphorylation of Thr-204 plays a key role in recognition and, subsequently, in activating plant defense responses. In this research we will determine all of the *Pto* amino acid residues that are phosphorylated and examine how these modifications affect the activity of the kinase. Our studies will be extended to investigate the role of Pto phosphorylation in the plant cell during interaction with the bacterial pathogen. Finally, to further understand recognition specificity of the Pto kinase we will isolate and characterize an additional *avrPto*-like gene from a *Pseudomonas* strain.

9901582 The Function and Evolution of Plant Myb Proteins

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Postdoctoral Fellowship; Grant 99-35301-8102; $90,000; 2 Years

Understanding the molecular mechanisms that control plant metabolism and form is fundamental to the use of genetic engineering to rationally manipulate specific features of crop plants. Because they can alter the expression of multiple genes, transcription factors (proteins that control gene expression) represent excellent targets for genetic manipulation. Myb proteins are members of a diverse family of plant transcription factors, and more than 100 distinct genes encoding Myb transcription factors are present in typical crop plants. Plant Myb proteins are related to a smaller family of animal transcription factors, but the plant and animal Myb proteins exhibit fundamental structural differences. Myb proteins from plants (designated pc-Myb proteins) that exhibit greater structural similarity to the Myb proteins present in animals than to the other Myb proteins in plants were identified recently. The identification of the novel pc-Myb proteins conserved between animals and plants and the existence of the diverse family of plant Myb proteins suggests that the smaller pc-Myb (and animal Myb) family represents an ancient group of proteins while the diverse plant Myb family represents a relatively recent innovation within the plants. The focus of this research will be to determine whether the ancient group of Myb proteins regulate a set of genes that are involved in fundamental cellular processes shared by plants and animals, and to examine whether specific plant Myb proteins regulate functions unique to plants. These results should provide information that may allow the use of some Myb genes in the genetic engineering of crop plants with the consequence significance for agriculture.

9901385 A Genetic Dissection of the Sex Determination Pathway in Maize

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Strengthening Award; Grant 99-35301-8048; $120,000; 2 Years

In maize, the sex of flowers is either male (the tassel) or female (the ear). However, at the initial stages of flower development, the tassel and the ear each contain both male and female structures. In the tassel, the female components degenerate by a process of cell death while in the ear, the male parts cease to develop by a process of cell arrest. Mutations called *tasselseed* exist, so named because the female organs do not die in the tassel but instead develop; hence, *the tassel contains seeds.* Two of the genes involved in the cell death process, *Tasselseed-1* and *Tasselseed-2* have been cloned and characterized. These two genes act in a programmed manner to cause the cells of female structures to die in the tassel and also in part of the ear. In addition to *tasselseed-1* and *tasselseed-2*, twenty-three new *tasselseed* mutations have been obtained from various sources. The purpose of these studies is to characterize these new mutations in terms of their effect on flower development and on cellular processes. The results of these studies will help us further understand the process of programmed cell death in maize. Unisexuality of flowers in maize provides for easy hybridization, a process important in commercial production of crops. Other cereal crops such as rice and wheat have bisexual flowers which hinders the process of hybridization. Our knowledge of unisexual flower development in maize will eventually provide the ability to develop unisexual lines of other cereal crops by genetic engineering.

9901343 A Grass Endophyte Locus Determining Expression of a Protective Alkaloid

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New Investigator Award; Grant 99-35301-8078; $120,000; 2 Years

Many forage, turf, woodland and meadow grasses host mutualistic fungal endophytes *(Epichloe* and *Neotyphodium* species) that are maternally transmitted (seed borne) and confer to the grasses profoundly enhanced resistance to biotic and abiotic stresses. Among the bioprotective alkaloids produced by endophytes are the lolines (saturated l-aminopyrrolizidines). These unique alkaloids are potent and broad-spectrum insecticides, and sometimes exceed 10,000 g/g plant dry weight. In addition to protection from insects, lolines may have other roles such as enhancing host drought tolerance. Genetic and molecular analyses can provide powerful tests of such roles. I have completed extensive genetic analysis of *Epichloe festucae*, and the results indicate a single genetic locus *(LOL)* that governs expression of lolines. Since I have also identified genetic markers associated with this locus, the next logical step is to construct a contiguous genetic and physical map spanning *LOL.* This map will facilitate development of biological materials necessary to directly test the roles of lolines in agricultural and ecological settings.

9901512 RNA Splicing and Turnover in Chloroplasts

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Grant 99-35301-7847; $130,000; 2 Years

Plants contain three different genomes: one each in the nuclear, chloroplast and mitochondrial compartments, respectively. Each of these genomes has a unique evolutionary history, and a distinct system for expressing those genes. Following the transcription of DNA into RNA, most RNAs have to be processed before they are functional. An interesting aspect of processing is "splicing", whereby intervening regions (i.e., introns) are removed, and the adjacent regions (i.e., exons) are spliced back together. Introns of the first two major groups, I and II, are common in chloroplast and mitochondrial genomes. Little is known about how these introns are efficiently spliced and regulated *in vivo*. We are studying the Group I introns in the chloroplast genome of *Chlamydomonas*, a powerful green algal model system for chloroplast molecular genetics. These introns are remarkable in that they can "self-splice" *in vitro* in the absence of proteins. However, evidence suggests that cellular factors facilitate splicing *in vivo*. Our proposed research is to identify such factors for the intron in the large ribosomal subunit (LSU) RNA using suppressor genetics. We have isolated one nuclear gene suppressor of a splicing- deficient version of the LSU intron, which we call *css1* (chloroplast splicing suppressor). We will genetically characterize *css1* further in order to clone the gene using complementation. Cellular levels of RNAs are also regulated by degradation rates. The second major goal of our proposed research is to elucidate the *in vivo* degradation pathway of the chloroplast *tufA* mRNA. This mRNA has a short half-life that is influenced by environmental and intrinsic factors. We have obtained novel preliminary data that provide a basis for determining the major elements regulating *tufA* mRNA stability *in vivo*. Overall, the proposed research will provide new, basic information on gene expression in plants, the understanding of which could promote the manipulation of genes for agricultural purposes.

9901504 Replication of the Plant Mitochondrial Genome

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Grant 99-35301-4428; $130,000; 2 Years

We propose to study the structure and mechanism of replication of plant mitochondrial DNA (mtDNA). Most plant mtDNA is found as complex structures (probably concatemers) larger than the genome size and as linear molecules, not as circular forms. We will obtain mtDNA from tobacco and liverwort cell cultures at various times during the growth cycle, including soon after subculture when replication is known to be highest and later as replication declines. Thus, we will monitor changes in the form and proportion of given mtDNA structures throughout the course of replication. Our methods will include pulse-labeling, digestion with endo- or exo-specific nucleases, and labeling the ends and single-stranded regions of the mtDNA molecules. Our specific objectives are: (1) To determine whether the linear molecules have a defined terminal sequence: a telomere. (2) To determine the arrangement of the genomic units within concatemeric mtDNA. We will then develop a model for plant mtDNA replication by comparison with mechanisms used to replicate linear DNAs from bacteria, bacteriophages, animal viruses and linear mtDNAs from yeasts. The data generated will be useful in choosing and designing mitochondrial genotypes for agricultural improvements, such as for cytoplasmic male sterility.

9901352 Regulation of Genetic Redundancy in Allotetraploid Hybrids of *Arabidopsis*

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Grant 99-35301-4429; $120,000; 2 Years

We propose to study changes in gene expression induced by allopolyploidization. Wheat, cotton and canola, which originated in prehistoric times, are successfully adapted allopolyploid hybrids. Allopolyploids can be synthesized by hybridization of two related species followed by chromosome doubling, to produce a compound genome made of two or more diploid sets of chromosomes. Comparison of newly synthesized allopolyploids with established ones suggests the evolution of several adaptations, although the molecular basis of none of them is known. One characteristic of synthetic allopolyploids involves extreme phenotypic variability, suggesting the silencing or modulation of gene activity, which could be caused by epigenetic interactions between homeologous genes. Our research using synthetic hybrids of *Arabidopsis thaliana* and *Cardaminopsis arenosa* demonstrated that gene silencing had occurred. Following up this observation, we will determine the pattern and extent of gene silencing by isolating a representative sample of silenced genes and studying their characteristics. Comparison of these silenced genes will probe their association with repeated DNA, a known cause of silencing. It will also assess the relationship between silencing and parental genomic origin, and the relationship between silencing and cytosine DNA methylation, which is often associated with epigenetic gene silencing. The information collected in this study will help us understand the forces that shape the evolution of allopolyploid hybrids. Because many important crops are allopolyploid, this study may contribute to the ability to better manipulate their genomes.

9901362 Genetic Analysis of Leaf Polarity in *Arabidopsis*

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Grant 99-35301-8049; $210,000; 3 Years

To provide a greater area for capturing sunlight, most plants make leaves with wide, flat blades. The upper half of the leaf blade is specialized for capturing light while the bottom half is specialized for gas exchange. In addition, the upper half of the leaf appears to possess a special ability to develop new buds which in turn grow out and become new branches. The production of leaves and branches is of obvious importance to the production of food crops. Our goal is to understand how leaf blades develop: how do the upper and lower halves become different from one another and what underlies the special ability of the upper half of the leaf to form new buds? Our research organism is the fast-growing, simple lab plant *Arabidopsis*. We will begin to understand this process by determining what type of protein the *PHABULOSA* gene encodes. The *PHABULOSA* gene, when mutated to a dominant form, causes all of the cells in the leaf to develop as though they are upper half cells, even those that would normally become lower half cells. We will also identify other genes that act to direct proper leaf blade development by finding mutations that enhance (make worse) or suppress (ameliorate) the *PHABULOSA* mutant defect. Such mutations are likely to be in genes that are required for the development of normal leaf polarity.

PLANT GROWTH AND DEVELOPMENT

Panel Manager - Dr. J. Clark Lagarias, University of California, Davis
Program Director - Dr. Liang-Shiou Lin

This program area supports research aimed at increasing our basic understanding of mechanisms underlying the regulation of plant growth and development in order to achieve optimal productivity of agriculturally important crop plants and forest species. Research areas emphasized by the program include: (1) mechanisms of cell division, expansion and differentiation, (2) development and organization of meristems, (3) response to environmental signals normally required for growth and development, (4) cell biology, including studies on cytoskeleton, membrane transport, protein trafficking, and cell wall structure and properties, (5) metabolism related to growth and development, (6) hormonal regulation of growth and development, (7) signal transduction mechanisms related to growth and development, and (8) analysis and control of growth patterns.

9901894 Functional Analysis of A Kinetochore-Associated Kinesin-Related Protein

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New Investigator Award; Grant 99-35304-8142; $150,000; 2 Years

Cell division is an essential process by which living organisms grow, develop, and reproduce. In flowering plants, cell division is the only way that certain organs develop to their mature shapes, because plant cells normally do not move around. Cell division must occur with high fidelity so that the genetic content gets maintained in a constant level among cells within a species. A fibrous protein network of microtubules is largely responsible for cell division to succeed. During cell division, microtubules organize into a dynamic array called the spindle apparatus that conducts the even segregation of genetic material-bearing chromosomes. Each replicated chromosome is attached to the spindle apparatus at its sibling kinetochores so that the sister chromatids will segregate equally into two daughter cells. In order to do so, microtubules undergo dynamic reorganization, which is believed to rely on a group of enzymes named motor proteins. They are kinesin and its related proteins and dyneins. To elucidate the mechanisms of plant cell division, we need to characterize these motor proteins that play crucial roles in this fundamentally important process. This proposal is aimed at characterizing one of the kinesinrelated proteins that plays a role in regulating the activity of chromosomes. We are going to determine the amino acid sequence of this protein in model plants rice and *Arabidopsis* by cloning the genes. In order to understand its function, we will also isolate this kinesin-related protein so that we can dissect its activities *in vitro.* Finally, we want to understand how this protein functions during cell division by taking cell biological approach. Information about the activities of the kinesin-related proteins will help us understand how plant cells divide so that ultimately it will help us understand the essence of plant growth and development. Such knowledge will be very useful for improving crop yields in the near future.

9901812 Role of Cotransporters in Water and Nitrogen Balance in Plants

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Water and nitrogen are essential for the growth and development of plants. How water is absorbed from the soil - to replace the water lost by transpiration in the leaves, and transported to different parts of plants is poorly understood. It is generally thought that water is transported across cell membranes by diffusion through membrane lipids, or through water channels, with the driving forces provided by hydrostatic or osmotic gradients. How the source of nitrogen (urea) is transported in plants is also not understood. We have recently found that water and urea are transported with nutrients (sugars and amino acids) by specialized membrane proteins. We will study the mechanism of transport by these "cotransporters", and assess their contribution to water and urea delivery across plant cell membranes, by expressing these cloned cotransporters in a model system, the *Xenopus* oocyte. We will simultaneously study the transport of nutrients, water and urea into the cells using state of the art biophysical techniques and microscopy. We will determine how much water and urea are carried by each protein, and characterize the molecular mechanisms of the water and urea fluxes. In addition, we will study the physiological role of transporters in water and urea balance using molecular biological methods by engineering plants lacking in specific transporters (in collaboration with Dr. Wolf Frommer, Institute for Botany, University of Tuebingen). The long-term benefit to agriculture will be to produce plants that can withstand varying environmental conditions, especially with respect to their water availability.

9901839 A Molecular Genetic Study of Floral Initiation of Plants in Response to Photoperiods

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Grant 99-35304-8085; $140,000; 2 Years

The flowering time of many plants is determined by the daily duration of light and/or darkness -- the photoperiod. Plants in which flowering occurs or is accelerated in long-day (LD) are referred to as LD plants which include many crop species. The molecular mechanism(s) determining plant flowering time in response to photoperiod is one of the fundamental questions in plant science and it is important to agricultural productivity. The genetics of plant flowering-time determination is best studied in the model plant *Arabidopsis* that is a facultative LD plant. However, the gene expression in *Arabidopsis* associated with floral induction needs to be investigated systematically in order to fully understand the process. We have shown previously that the blue-light receptor CRY2 plays an important role in *Arabidopsis* flowering-time regulation in response to photoperiods, and that CRY2 exerts its function via the control of gene expression. We propose to use two complementary approaches to identify and characterize genes that are regulated by CRY2. The first approach involves the use of differential display to identify genes for which the expression is affected by the conditional nuclear targeting of the CRY2 protein. The second approach is to identify genes for which the expression is altered by the mutation or overexpression of *CRY2*, at the genomic scale, using DNA micro array technology. The functions of those genes identified in our study will be further studied using conventional molecular genetic methodologies to elucidate their functions in plant flowering time determination.

9901844 The Role of Protein Kinase CK2 in Circadian Rhythms

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Grant 99-35304-8156; $240,000; 3 Years

Many biochemical and physiological processes in plants display endogenous rhythms with a periodicity close to 24 hours; thus, these rhythms have become known as circadian rhythms. Such rhythms are controlled by an internal time keeping mechanism called a circadian clock. This biological clock confers the ability to organisms to time various biological functions and to anticipate the coming of dawn or dusk. We have identified some of the molecules that are involved in circadian rhythms in plants, and one of these is a protein kinase called CK2. We plan to investigate exactly what role this regulatory enzyme plays in circadian rhythms by examining its potential substrates in the plant. We know that it can interact with and phosphorylate two other clock-associated proteins, and we will try to determine the function of this phosphorylation in the plant. We will also address the question of how the activity of CK2 on its substrates might be regulated. Because circadian rhythms are involved in many aspects of plant growth and metabolism, this research will have obvious practical implications for agriculture, including the possibility of regulating the flowering time of crop plants.

9901819 Cell Wall Digestive Enzymes in the Anthers and on the Pollen of Maize

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Grant 99-35304-8019; $246,000; 3 Years

Modulating sexual reproduction in crops is important to productivity. Promoting sexual reproduction could enhance the yield of fruits and seeds. Suppressing the process could increase the production of vegetable crops. Other possible manipulations include utilizing male sterile genes in the production of hybrid seeds, generating seedless fruits, and eliminating self-incompatibility. A major step in sexual reproduction is the interaction between the pollen (male sex cells) and female constituent (containing the eggs) of the flower. An essential component in the interaction is the pollen-surface materials, most of which are initially synthesized in the cells surrounding the pollen sac in the male component (anther) of the flower, where the pollen mature. Recent studies with insect/self-pollinated species have enhanced our understanding of the nature of the pollen surface materials and their interaction with the female constituent of the flower. However, we know little about the system in wind-pollinated species such as cereals, even though corn and its hybrid seeds are very important in American agriculture. The project studies the interaction among the cells surrounding the pollen sac, the pollen surface, and the female constituent in the flowers of corn . The synthesis and secretion of the digestive enzymes in the cells surrounding the pollen sac and their deposition onto the pollen surface will be probed. The physiological function of these digestive enzymes in hydrolyzing the cell wall of the female constituent of the flower for the penetration of the pollen tube and in aiding hydration of the pollen for germination and tube growth will be investigated.

9901899 Molecular Mechanisms of Leaf Blade Development

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Strengthening Award; Grant 99-35304-8193; $100,000; 2 Years

Genes controlling the basic patterns of plant growth and development (shorter stature, earlier maturity, and bush vs. vine architecture) have been used extensively by breeders to tailor crop varieties for specific environmental niches. Research on cloning and molecular analysis of the genes involved will allow us to make these modifications more efficiently through genetic engineering of existing strains. This improves our response time to new agricultural problems and enhances the competitiveness of our domestic agricultural system. Our research is focused on genes controlling leaf development, using diploid *Nicotiana* as a model system. We recently isolated the *NTPHAN* gene from *Nicotiana tabacum* which is involved in leaf initiation and development of the leaf blade. Analysis of the DNA sequence shows a high degree of homology to the corresponding *PHAN* genes of maize, *Antirrhinum* and *Arabidopsis* indicating conservation of this function in a range of distantly related plants. We are investigating the function of this gene (1) by disrupting its expression with an antisense *NTPHAN* transgene, and (2) by extending its expression with a sense *NTPHAN* transgene. Results from antisense disruption indicate that *NTPHAN* is essential for proper development of photosynthetic cell layers near the upper surface of the leaf. Sense over expression of *NTPHAN* appears to produce a reiteration of photosynthetic cell layers on the under surface of the leaf. Our current work is aimed at elucidating its normal expression pattern in the leaf and investigation of *KNOX* genes as potential targets of *NTPHAN* regulation.

9901870 Molecular Interactions During Import of Thylakoid Proteins

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Strengthening Award; Grant 99-35304-8095; $120,000; 2 Years

The long range improvement and sustainable productivity of agriculturally important crop and forest plant species require a fundamental understanding of processes that are critical to the development and vitality of these species. This includes understanding the mechanisms involved in protein trafficking within plant cells. All proteins must be correctly targeted and localized in order to perform their functions at optimal levels. Thylakoid proteins, which serve in the conversion of light energy to chemical energy and in the process of evolving oxygen, are instrumental to the growth of the plant. Yet these proteins are correctly routed to the thylakoid against several odds: most are nuclear-encoded, must cross multiple intraorganellar compartments, and they must be specifically recognized by one of at least four different thylakoid transport pathways in operation. The goals of our studies are to elucidate how proteins are specifically recognized and transported by the Sec pathway and distinguished from proteins that utilize other thylakoid transport systems. CPSecA is a key component which may target proteins and initiate membrane translocation in the Sec pathway via recognition of their amino-terminal signal peptides. We will study the specificity of the interaction of CPSecA with signal peptides using *in vitro* biochemical assays and purified components. The molecular outcome of this interaction will be evaluated in terms of changes in the ATPase activity, stability, and conformation of CPSecA. Understanding how thylakoid proteins utilize the proper transport pathway to achieve their final destination successfully and efficiently is an important step toward elucidating mechanisms for enhancing vigorous plant life.

9901889 Mechanism of Light and Developmental Control of Gene Expression in Arabidopsis

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Grant 99-35304-8641; $160,000; 2 Years

Higher plants possess a sophisticated signaling network to sense the light environment around them and modify growth for optimal use of available light energy. Many light-modulated plant responses have been shown to involve light regulated gene expression. Previous molecular and genetic studies have been shown that the *Arabidopsis* transcription factor HY5 likely represents a key transcription factor that plays a critical role in light control of gene expression. How light signals regulate the activity of HY5 will the focus of this proposed study.

We have shown that HY5 accumulation is light dependent. I propose to first determine which step(s) of HY5 expression (transcription to protein degradation) is subject to light regulation. We will determine the specific roles of the well-defined photo receptors in regulating HY5 accumulation by examining the HY5 accumulation pattern in selected null photo receptor single or double mutant strains under different light conditions. Our preliminary studies suggested that HY5 is present as a 160 kD protein complex in plants. I propose to further characterize potentially important protein complex through direct biochemical purification of the HY5 complex. The HY5 complex purification will be achieved by combination of conventional biochemical steps and affinity purification using specific HY5 antibodies. Our goal is to reveal the molecular identities of the HY5-associated proteins and to understand the mode of action of HY5. Accomplishment of this proposed work will be a significant step forward in our understanding of molecular mechanism for light regulated gene

expression. It will be vitally important for the ultimate goal of developing genetically superior or desirable varieties of crop and forest species.

9901818 Genetic and Molecular Characterization of *Semaphore1* Gene Function in Maize

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Grant 99-35304-8096; $140,000; 2 Years

Our ultimate goal is to understand the molecular mechanisms of leaf development, a developmental process that is crucial to the vitality of crop plants. The vegetative lateral organs (leaves and stems) of plants are formed from discrete, developmental structures called shoot apical meristem (SAMs), which are borne at the tips of growing plants. SAMs are responsible for the development of the entire above ground components (shoots) of the plant. SAMs are distinguishable from lateral organs at the molecular level by the differential expression of *KNOX* genes. *KNOX* genes are expressed at very high levels in SAMs, but not in leaves. Therefore, correct *KNOX* gene expression is crucial to the process whereby plant cells comprising the indeterminate SAM are programmed to develop into specialized, determinate organs such as leaves. This fundamental process, whereby plants make leaves, is poorly understood. The aim of this project is to utilize a new mutation identified in corn, called *semaphore1*, in order to elucidate the role of *KNOX* gene expression during shoot development. The *Semaphore1* gene is required in order to restrict *KNOX* gene expression to SAMs; plants harboring the *semaphore1* mutation exhibit abnormal expression patterns of *KNOX* genes. In contrast to normal plants, semaphore plants accumulate *KNOX* gene products in leaves and consequently display extreme defects in the development of leaves and stems. Genetic and molecular experiments are designed to determine the structure of the *Semaphore1* gene and its specific role in the regulation of *KNOX* gene expression during leaf development.

9901803 Molecular and Genetic Analysis of Fiber Differentiation

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Grant 99-35304-8093; $160,000; 3 Years

Fiber cells with thickened secondary walls provide the strength to support the heavy plant body and serve as a physical barrier against herbivore attack. Physiological studies have shown that fiber cells are induced along the path of auxin flow, and three plant hormones auxin, cytokinin and gibberellic acid are required for fiber differentiation. However, it is not known how fiber differentiation is spatially controlled and how the hormonal signals are perceived and transduced to induce fiber formation. We have initiated a genetic approach to study the mechanisms controlling fiber differentiation. Using the model plant *Arabidopsis*, we have isolated an *ifl1* mutant which lacks normal interfascicular fibers in the inflorescence stems. We have successfully fine-mapped the *ifl1* locus and cloned the *IFL1* gene. Furthermore, we have found that the auxin polar transport activity in the *ifl1* stems was dramatically reduced. This result directly links the *IFL1* function to the physiological studies regarding the role of auxin flow in fiber differentiation. It also suggests that *IFL1* might be involved in the spatial control of auxin flow path for fiber differentiation. These new findings provide us an unprecedented opportunity to further study the mechanisms on the spatial control of fiber differentiation. We will further investigate the role of *IFL1* in auxin polar transport and fiber differentiation, examine the *IFL1* expression pattern, investigate the function of *IFL1* by over expression of *IFL1* and its domains, and search for genes regulated by *IFL1*. Because fibers produced by plants have many economic uses such as in paper making and textiles, further understanding the underlying mechanisms of fiber differentiation not only would increase our knowledge of basic scientific aspects of differentiation, but also would have economic and agronomic implications.

9901794 Starch Debranching Enzyme Activity in an Allelic Series of *Sugaryl* Mutants

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Grant 99-35304-8642; $240,000; 3 Years

This project investigates the mechanisms of starch biosynthesis in plants. Starch granules are of major interest in agricultural research because they are the most important source of calories in the human diet, and because they provide raw material for many industries. Amylopectin is the glucose polymer in starch that is of primary importance for the properties and applications of granules. Glucose groups in amylopectin are connected to form relatively short "linear" chains, and these are attached to each other by "branch" linkages to form an extensive network. Amylopectin is very architecturally specific, because branches are not randomly located, but instead are found in a regular, repeating pattern. The biosynthetic mechanisms that impart this architectural specificity are not well understood, and these are the subject of this research proposal. The project investigates the role of debranching enzymes (DBEs) in determining amylopectin architecture. Altering DBEs causes large changes in starch structure, but their specific role in the biosynthetic process is not known. The general approach to this question is to systematically correlate starch structural changes with different types of alterations in DBE function. A collection of mutant maize lines with different DBE activities, caused by different mutations in the *sugaryl*

gene, has been established. Each mutant will be characterized in detail regarding the structure of the starch it produces and also the abundance and activity of the two major DBEs present. By comparing these activity levels with specific structural changes, the features of starch that depend on DBE activity will be evident.

9901881 Blue Light Regulation

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Grant 99-35304-8192; $160,000; 2 Years

The long-term goal of this project is to define the biochemical pathway used by blue light to induce transcription of specific nuclear-coded genes. Our current efforts are focused on expression of specific members of the LHCB1 (light harvesting chlorophyll binding) gene family in pea and *Arabidopsis* whose expression is regulated by blue light. Transcription of these genes is triggered by a single pulse of blue light at fluences comparable to one second of moonlight. Because of this sensitivity, the blue light system regulating these events has been called the blue low fluence system. The blue low fluence system is independent of the well characterized phytochrome system and the recently characterized blue light systems regulating phototropic curvature and stem length. Transgenic analysis of these genes very strongly suggests that the sequence element necessary for this response is an ACT/CAATT box found in the LHCB genes regulated by blue light. The same sequence is absent in unregulated LHC genes. Excitation of the BLF-system is known to activate a heterotrimeric G protein. Preliminary data from G antisense expressing plants strongly suggests a role for the *Arabidopsis* protein in blue light mediated LHCB1 transcription. The two immediate goals of this proposal are: 1) To confirm the necessary and sufficient role of the ACT/CAATT box in the blue low fluence response; and 2) To define the role of heterotrimeric GTP binding proteins in the BLF response.

9901834 Identification and Characterization of Partners of Profilin from Pollen

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Grant 99-35304-8640; $240,000; 3 Years

Plant reproduction depends upon the successful transmission of sperm to the embryo sac, where double-fertilization occurs. Sperm from angiosperms are non-motile, but are deposited in the embryo sac through the unique behavior of the vegetative cell of the pollen grain. Polarization of cytoplasm, selection of a germination site and pollen tube extension are all dependent upon the dynamic behavior of a filamentous actin cytoskeleton. Profilin is a complex regulator of actin organization that can cause disassembly or promote polymerization and has been identified as an abundant component of plant pollen. The biochemical characterization of pollen profilin reveals that it may be a key intermediate between signal transduction cascades and actin organization. Surprisingly, multiple profilin isoforms from maize pollen differ in the ability to alter cytoplasmic architecture. These differences correlate with the ability to bind to poly-L-proline *in vitro*, which presumably reflects the ability to bind proline-rich cellular proteins. Our hypothesis is that profilin is a major regulator of pollen actin organization and that profilin isoforms perform distinct functions during pollen development. Further, we predict that profilin activity is modulated by interaction with specific proteins, which we term partners of profilin from pollen (POPPs). Experiments are designed to identify and characterize the function of POPPs from maize pollen. By these studies we will provide critical insight into the mechanisms used to control profilin function and thereby to regulate actin organization and dynamics. The findings will dramatically improve knowledge of pollen biology and provide tools for future manipulation of plant sexual reproduction.

9901886 Regulation of F-Actin During Last Epidermal Development in *Arabidopsis*

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New Investigator Award; Grant 99-35304-8525; $216,000; 3 Years

The growth of plant cells depends on inputs from the environment such as temperature, humidity, and light. Growth also depends on intrinsic signals such as nutrient availability and hormone signals. The question of how plant cells translate these diverse types of signals into a coordinated growth response is an important question in plant biology. Although much progress has been made in identifying many of the genes that perceive growth signals, little is known about how these signals are transmitted to downstream components such as the cytoskeleton to control cell growth. The cytoskeleton consists of filamentous arrays that regulate both the delivery of construction materials to the cell wall and the direction in which the cell expands. For example, the actin cytoskeleton forms a 3-dimensional network of "tracks" on which vesicles containing construction materials can travel. The actin tracks are very dynamic, and the arrangement of filaments can change in response to hormone signals, pathogen attack, and light. A mechanistic understanding of how actin organization is controlled is essential to understand how plant cells grow and improve our ability to genetically engineer complex traits. An interdisciplinary approach using *Arabidopsis* epidermal development has been adopted to identify genes that regulate actin organization and growth. This proposal is centered on understanding the function of a transmembrane

protein that is hypothesized to regulate cytoskeletal organization during cell elongation. It is anticipated that a careful examination of the *F-ACTIN ALTERED* gene will lead to the identification of additional players in the cytoskeletal growth control pathway.

9901795 Characterization of Plasma Membrane F-actin Binding Proteins
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Strengthening Award; Grant 99-35304-8009; $138,000; 2 Years

The actin cytoskeleton is a spatially and temporally dynamic protein filament-based system which functions in a number of essential processes in all eukaryotic cells. In plant cells actin cytoskeletal dynamics are involved in a number of cellular processes which are critical for normal plant growth, development and thereby productivity. To meet the multiple demands for the actin cytoskeleton during the life of the cell, actin filaments participate in an elaborate choreography which involves their assembly, disassembly and association with other proteins to form arrays specialized for particular functions. Actin-binding proteins play an important role in regulating this dynamic behavior of the actin cytoskeleton. The goal of this research is to purify novel plant plasma membrane F-actin-binding proteins and then develop the molecular tools required to study their cellular functions. The specific objectives are to 1) purify plasma membrane-associated F-actin-binding proteins which have been identified using a F-actin gel overlay assay, 2) obtain the corresponding clones, 3) produce antibodies to these proteins and, 4) determine the cellular and subcellular location of these proteins. Completion of these experiments will contribute to our understanding of fundamental cellular processes which are essential for optimal plant growth, development and reproduction.

9901830 Expansin Expression During Adventitious Rooting in Loblolly Pine
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Strengthening Award; Grant 99-35304-8115; $120,000; 2 Years

One of the most significant changes that occurs as conifers age is the loss of the ability of cuttings to form roots. In loblolly pine seedlings, a gradient in root forming ability is evident early in seedling development. While the primary root will readily form lateral roots, cuttings in the base of the stem (hypocotyl) will only form roots in response to the application of the plant hormone auxin. Cuttings in the upper part of the stem (epicotyl) form few, if any roots, regardless of whether auxin has been applied or not. We have determined that a gene family called expansin was expressed in loblolly pine hypocotyl cuttings during the first 12 to 24h after the application of auxin. Expansins are responsible for the loosening of primary cell walls, a necessary step for changes in development. We have also determined that expansin is expressed in cells from which roots will be formed. These data suggest that expansin is an essential early event in the formation of roots. To continue this work we plan to determine the genetic structure and the expression patterns of the individual members of the expansin gene family in loblolly pine from both rooting and non-rooting tissue. In addition, in other plants the direct application of expansin protein has been shown to effect development. We will also test this ability of expansin by direct application of the expansin protein to pine seedlings. Our expectation is that it will induce at least some of the necessary changes for root formation.

9901800 Photoperiodic Control of Gibberellin Metabolism in Long-Day Rosette Plants
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Grant 97-35304-4692; $132,000; 2 Years

Long-day rosette plants, such as lettuce and spinach, grow as rosettes (no stems) during the short days of winter. They produce stems and flowers (bolt) during late spring and early summer when the days are long. Previous work has shown that production of gibberellins, a class of plant hormones, is promoted by long days and that an increase in gibberellins is essential for bolting. The objective of the proposed research is to learn how day length regulates gibberellin production in connection with bolting in rosette plants. Rate-limiting steps for the production of gibberellins will be determined in spinach and a rosette tobacco. In these plants, the genes for gibberellin 20-oxidase and 3d-hydroxylase will be over- or under-expressed, which is expected to result in an increase or decrease in active gibberellin, respectively. We also plan to clone the gene for gibberellin 2-oxidase and investigate its expression under various photoperiodic conditions. Gibberellin 2-oxidase is of interest, because it deactivates active gibberellins and thus can prevent bolting in rosette plants. The proposed work will provide basic knowledge of how long summer days stimulate gibberellin production, leading to an increase in the level of active gibberellin, which in turn causes bolting in rosette plants. This information will provide the basis for genetic manipulation of growth and development, especially of stature, in agricultural plants.

9901898 The Evolution of Floral Homeotic Gene Expression
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Postdoctoral Fellowship; Grant 99-35304-8131; $88,000; 2 Years

The evolutionary innovation of the angiosperm flower sparked a great radiation, establishing angiosperms as the dominant plant group on earth. Yet little is known about flower origins. Recent studies in plant development have established the roles of several genes involved in floral development, providing a new tool for the study of the evolution of gene expression. Gnetales, the sister group to angiosperms, have many characteristics otherwise restricted to the angiosperms, including flower-like reproductive units. This proposal will address the evolution of the flower by determining the expression patterns of floral homeotic genes in the gnetophyte *Welwitschia*. Degenerate PCR primers will amplify regions of homologous genes, which will be used as probes to isolate full-length clones from genomic and cDNA libraries. Nucleotide and inferred protein sequences will be compared to the sequences of homologous genes to determine changes that have occurred over evolutionary time. *In situ* hybridizations will determine the spatial expression patterns of these genes and establish homology to floral organs. Northern hybridizations on dissected reproductive units and other tissues will determine the relative levels of gene expression. This study addresses the origin of flowers, the evolution of gene expression, and the molecular-developmental basis of morphological change.

9901864 Genetic and Biochemical Analysis of Phototropic Signal Transduction in *Arabidopsis thaliana*

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Grant 99-35304-8060; $240,000; 3 Years

Plants are dependent upon light not only as an energy source to drive photosynthesis, but also as a modulator of growth and development. Multiple components of sunlight reaching the earth's surface are used by plants as regulator`y cues through the action of several photo receptor systems: including the red/far-red light-absorbing phytochromes, blue/ UV-A light-absorbing photo receptors, and UV-A/UV -B light-absorbing photo receptors. Although much is known about the nature of the phytochrome family of photoreceptors, little is known about other plant photo receptor systems. It is also largely unknown how any of these classes of photo receptors once activated transduce signals to their appropriate sites of action. In this proposal we will use the small plant, *Arabidopsis Thaliana* (mouse ear cress), as a model system to investigate the genetic and molecular basis for one of the signal transduction events mediating phototropism - or the bending response various plant organs elicit in response to directional light cues. Specifically we propose to use mutants we have isolated that disrupt phototropism to characterize the phototropic response (as well as other photobiological responses), and molecular biochemical approaches to study the function of the NPH3 protein encodecl by one of the genes identified via mutation. The research proposed here should provide a wealth of information about the mechanisms required for phototropic responses of plants and thus provide valuable information that can be applied to agronomic plant species to potentially increase productivity.

9901880 Molecular Cloning of *STUNTED PLANT-1*, a Gene Modulating Cytokinin Response

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Postdoctoral Fellowship; Grant 99-35304-8141; $90,000; 2 Years

Plant hormones are chemical compounds produced in plants which direct growth and development. Understanding how hormones achieve their effects in plants provides us with knowledge which can be used to alter how plants grow and develop, and thus improve agriculture. One important class of hormones in plants are the cytokinins. Cytokinins play a role in a multitude of processes, and are known to promote cell division, redirect the distribution of metabolic resources, alter the overall form of the plant, and increase lifespan by inhibiting senescence. However, the mechanisms by which cytokinins achieve these effects are poorly understood. To study the mechanism of cytokinin action, we propose to use the *Arabidopsis thaliana* mutant *stunted plant-1 (stp1).* The mutant has multiple physiological characteristics that are consistent with elevated cytokinin sensitivity. The project will characterize the mutant in three ways: physiologically, genetically, and by cloning the *STP1* gene. By understanding the nature of the mutated gene, it will be possible to reconstruct the normal processes involved in the plant's cytokinin perception and response. This will provide knowledge that will be used to develop a model for the mechanisms by which cytokinins work in plants.

9901814 Regulation of the Gibberellin Biosynthetic Genes *GA1* and *GA2* in *Arabidopsis*
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Grant 99-35304-8061; $150,000; 2 Years

Bioactive gibberellins (GAs) are plant growth regulators, which control such diverse processes as germination, stem elongation, and flower and fruit development. The first committed step in GA biosynthetic pathway is catalyzed by two cyclases, which are encoded by the *GA1* and *GA2* genes in *Arabidopsis*. This proposal describes experiments designed to investigate the effect of overexpression of these two genes on plant growth and development. The second goal is to identify factors that regulate the *GA1* gene expression by characterizing *Arabidopsis* mutants with altered *GA1* expression. These studies will be important to improve crops by manipulating GA levels in specific tissues to control their growth.

9901797 Molecular Analysis of Brassinosteroid Signal Transduction
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Grant 99-35304-8059; $140,000; 2 Years

A great deal is known concerning the molecular mechanisms by which steroid hormones regulate gene expression in vertebrates and insects. In contrast, the growth-promoting steroids found in higher plants are only beginning to be studied in depth. Brassinosteroids are plant steroid hormones that act at low concentrations (one billionth of a gram) to profoundly affect the growth of a wide range of plants including *Arabidopsis*, corn, soybean, rice, potatoes, radish, lettuce, pepper and tomato. With previous USDA support, our laboratory showed that brassinosteroids enhanced the elongation of stems and regulated gene expression in plants. We also isolated the first brassinosteroid-insensitive mutant, which is a valuable tool in understanding the mechanism of action of these compounds. The objective of the current USDA grant is to study how cells perceive brassinosteroid at the cell surface, and how this then results in the regulation of genes that are responsible for controlling aspects of plant growth and development. To do this we will employ the techniques of molecular genetics and biochemistry, using cloned genes, synthetic proteins and labeled brassinosteroids. Because brassinosteroids are similar in structure to animal steroid hormones, understanding their activity has great potential importance in basic developmental biology. In view of the multitude of demonstrated effects brassinosteroids have on crops, a deeper understanding of the molecular mechanisms of brassinosteroid action would have agricultural potential as well.

9901808 Research Conference 'Auxin 2000'
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Grant 99-35304-8001; $6,000; 1 Year

The plant hormone auxin is ubiquitous among plant species and has a key role in most aspects of plant growth and development, including those that are directly related to crop performance. During the last few years, the application of new analytical, biochemical and genetic approaches has led to dramatic advances in our understanding of auxin metabolism, transport and action. At the same time, the availability of the complete genome sequence of *Arabidopsis*, and perhaps other, plant species within the next few years will accelerate the rate of discovery. For these reasons, we are planning an international meeting of biologists working on all aspects of auxin biology. The meeting, the first of its kind and to be named "Auxin 2000", will be held in May 2000 on the island of Corsica. Our objective is to bring the very best researchers in the field together in an atmosphere conducive to formal presentation of the latest research results as well as informal discussion and consultation. To promote interaction, attendance at the meeting will be limited to 120 scientists. Approximately 45 of the attendees will be invited speakers. The remainder will be selected by the organizers from a pool of applicants. Approximately 40 places will be reserved for postdocs and senior graduate students. We request funds for a small fraction of the travel cost of the invited US speakers and for fellowships to US postdocs and senior graduate students to participate.

9901793 Molecular and Developmental Analysis of *fasciated ear* Mutants in Maize
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Grant 99-35304-8058; $210,000; 2 Years

Plant morphology is a major factor determining crop yield. The growth of plant shoots is indeterminate and is controlled by the meristems, groups of stem cells that initiate leaves and flowers during the life of the plant. The long term objective of this proposal is to understand how the meristem functions and how the size of the meristem is controlled. We have isolated a class of mutants, called *fasciated ear* (*fae*), that affect the size of the maize ear inflorescence meristem. These mutants will be carefully characterized using cellular and molecular techniques to understand how the corresponding genes control meristem size. For one of the mutants, *fae2*, we have isolated a candidate clone using a transposon tag, and we will determine if this clone corresponds to the *fae2* gene and study its expression pattern. This will help us to understand

how *fae2* controls meristem size and the shape of the maize ear. This advance in our understanding of the molecular mechanisms controlling meristem function could help improve yield in maize and other crop plants.

9901877 Role of the *FOUR LIPS* Gene Product in Stomatal Differentiation and Cell Cycle Regulation

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Grant 99-35304-8098; $210,000; 3 Years

Stomata consist of two guard cells that surround a pore that regulates gas exchange for photosynthesis. Although stomata are crucial for crop and plant productivity, little is known about the genes needed for stomatal development. As part of a long-term study of the molecular genetics of stomatal development in the model plant *Arabidopsis*, we have identified mutations at the *FOUR LIPS (FLP)* locus that result in stomata in contact. Clusters of stomata are almost never found in wild-type plants. In wild-type plants, stomata develop from a guard mother cell (GMC) which divides symmetrically to produce two guard cells. Existing data indicate that the stomatal clusters in *flp* develop from extra generations of guard mother cells resulting in cell duplications. This would explain the morphology of the clusters where the stomata are arranged side by side. Together these data suggest that the *FLP* gene product is somehow involved either in regulating the timing of development or in controlling the division machinery. Thus *FLP* may act at the intersection of cell differentiation and cell cycling, an area that is poorly understood in plants. The major goals of this work are to determine the molecular identity and function(s) of the *FLP* gene product by (1) cloning the gene, (2) altering the level of gene expression and analyzing the phenotypic consequences, and (3) studying the cytology of wild-type and mutant plants. The identification of genes controlling stomatal formation should provide opportunities for genetically engineering new traits in crop plants related to yield, drought tolerance and pathogen entry.

9901816 Flowering Plant Fertilization: Attraction, Interaction and Transport of Gametes

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Grant 99-35304-8097; $150,000; 2 Years

Flowering plants account for essentially all crops, yet relatively little is known about their sexual interaction at the gamete level. My work uses two species as fertilization models in flowering plants—tobacco (*Nicotiana tabacum*) and the leadwort (*Plumbago zeylanica*)—among the most extensively studied plants for this topic. In contrast to animals, sperm cells of flowering plants occur in pairs (rather than clouds of millions), are non-motile in their transport, and the two sperm cells participate in separate fertilization events, forming the zygote (and subsequent embryo) and the polyploid nutritive tissue known as the endosperm—a process known as double fertilization. This study concentrates on the interactions and attraction of male and female gametes. *Nicotiana* is more typical of a normal flowering plant since it contains the full complement of cells in the embryo sac, where the egg cell and fertilization occurs. In *Plumbago*, the two sperm cells differ enough that their eventual fusion fate has been identified and characterized. The smaller of the two sperm cells, which fuses with the egg cell, contains organelles that may become chloroplasts. The larger of the two sperm cells fuses with the central cell to form the nutritive endosperm. The basis of this preferential fertilization, how the sperm cells move, and the molecular basis of their attraction is part of the current study. Immunocytochemical, electrophoretic and biochemical experiments will be done to elucidate how sperm cells are selected and interact with the molecules that propel them within the pollen tube and embryo sac.

9901847 Role of Petunia Receptor-Like Kinase PRK1 in Pollen Development

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Grant 99-35304-8004; $150,000; 2 Years

We have previously identified a receptor-like kinase of petunia, named PRK1, which is produced predominantly in pollen, and shown that it is required for the progression of microspores from the unicellular stage to the bicellular stage during pollen development. We have further used a protein-protein interaction assay carried out in yeast to identify a pollen protein, named KIP1, that interacts with the kinase domain of PRK1. Using the same assay, we have shown that KIP1 interacts with itself. In this project, we will identify the regions of KIP1 that interact with PRK1 and with itself; use several *in vitro* methods to determine whether recombinant PRK1 and KIP1 produced in *E. coli* or yeast interact with each other; ascertain the physiological relevance of the PRK1/KIP1 interaction by determining whether transgenic plants not producing KIP1 or producing a truncated KIP1 show similar pollen development phenotypes as transgenic plants not producing PRK1; and determine whether the kinase activity of PRK1 is essential for its function by examining whether production of a mutant form of PRK1 lacking kinase activity has any dominant-negative effect on pollen development in transgenic plants. The information gained from this project will likely contribute to a better understanding of the molecular and biochemical mechanisms of signal transduction events that occur during pollen development. On the practical side, this knowledge will

make it more feasible to disrupt a specific stage of pollen development for the generation of male sterile plants to facilitate hybrid seed production, a very important agricultural practice.

9901895 MRP Subclass ABC Transporters from *Arabidopsis*

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Grant 99-35304-8094; $240,000; 3 Years

This project is based on our recent success in the isolation of two genes, *AtMRPI* and *AtMRP2,* from the model plant *Arabidopsis thaliana.* These genes encode membrane proteins belonging to the ATP-binding cassette (ABC) superfamily of transporters. Having established that AtMRP1 and AtMRP2 transport a broad range of toxic compounds, including glutathionated herbicides, pigments and antimicrobial agents *in vitro,* our next objective is to define what these transporters and other members of the AtMRP subclass of ABC transporters do *in vivo* and whether some might also transport heavy metals. Specific aims will include: (i) Determination of the membranes with which AtMRP1 and AtMRP2 are most closely associated to learn if, as we suspect, they deliver their transport substrates into the vacuole. (ii) Tests of the capacity of AtMRP3, the only AtMRP known to confer resistance to cadmium salts when expressed in yeast, for the transport of organometal complexes. (iii) Analysis of the physiology of plants lacking functional *AtMRPI* and/or *AtMRP2* genes to examine the involvement of these genes in xenobiotic (primarily herbicide) detoxification and protection from oxidative stress in the intact plant. An increase in our understanding of the mechanism of action and roles played by this recently discovered class of plant transporters may yield new strategies for manipulating plant resistance to various toxins, including herbicides and possibly heavy metals.

9901801 Peptide Transport in *Arabidopsis thaliana*

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Grant 99-35304-8194; $160,000; 2 Years

Peptide transport is a widespread phenomenon exemplified by the translocation of peptides 2-6 residues in length across the plasma membrane in an energy dependent and saturable manner. Peptide transport systems are distinct from amino acid uptake and play a variety of roles in bacteria to man. Peptide transporters fall into three families; the ATP binding cassette family (ABC transporters), the peptide transporter (PTR) family (transporting di- and tripeptides) and the recently described oligopeptide transporter (OPT) family. We are focusing our efforts on members of the PTR and OPT family found in the model plant, *Arabidopsis thaliana.* Our data suggest that these peptide transporters may play a role in normal growth and development by transporting peptides that act as signals. We intend to test this hypothesis by creating transgenic plants defective in the expression of the transporters. We will also isolate mutants that are defective in transport. This work, coupled with expression studies to look at function, will allow us to better define the role of these transporters in the plant. Although well studied in animal systems, the idea that peptides could serve as signals in plants is largely unexplored. Our efforts represent one avenue to investigate the role of peptides in plants.

9901853 Regulation of *N*-Acylethanolamine Metabolism in Seeds

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Grant 99-35304-8002; $142,000; 2 Years

N-Acylphosphatidylethanolamine (NAPE) is metabolized by phospholipase D (PLD) to yield phosphatidic and *N*-acylethanolamine (NAE), and evidence indicates that this pathway is responsible for the signal-mediated release of endogenous cannabinoid lipid mediators in neuronal and other vertebrate cell types. Here we propose to continue our work with NAPE metabolism in plants, now focusing on the catabolism of both NAPE and NAE in seeds. Our aim is to quantify NAE molecular species and to correlate the levels of this metabolite with the activities of the enzymes likely to regulate its levels *in vivo* (PLD / and NAE amidohydrolase). This will be accomplished in the physiological context of seed development, germination and post-germinative seedling growth, where we have acquired considerable biochemical data on the biosynthesis of NAPE. In support of our goal, we have developed accurate analytical procedures that reproducibly quantify trace levels of NAE molecular species in crude plant lipid extracts. Moreover, we have developed a new assay for the detection of NAE amidohydrolase enzyme(s) *in vitro* and identified a novel NAE 18:2 hydrolyzing activity in extracts of imbibed cottonseeds. The proposed research will help to identify new bioactive lipids in seeds with possible cannabimimetic properties. More importantly, our work will form the basis for defining the endogenous role of NAPE/NAE metabolism in seed development, germination and seedling growth, which perhaps is an evolutionarily conserved signal transduction pathway present in all multicellular eukaryotes.

9901796 The Biochemistry of Glycosylphosphatidylinositol-anchored Proteins in Plants

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Grant 99-35304-8003; $140,000; 2 Years

The project will study a relatively newly-discovered way by which certain proteins are attached to the outer surface of plant cells. These proteins are bonded to a chain of four sugar molecules terminating in a single phospholipid molecule, which holds the protein tightly in the cell surface membrane. These proteins, termed glycosylphosphatidylinositol (GPI)-anchored proteins, are known to be widespread in animal cells but are only now being found in plants. In animals different GPI-anchored proteins function as receptors for growth substances, for cell-cell interactions, and for protection against potentially damaging external molecules. It now appears that they are equally important to plants. GPI-anchored proteins are involved in the uptake and utilization of phosphate and nitrate, two nutrients critical for plant growth. Phosphate availability is often the factor which limits the rate of plant growth. Very recently it was also discovered that one of the most abundant glycoproteins of growing plant cell walls is GPI-anchored. The physiological significance of this finding is not yet apparent. A major goal of this project is to learn how the GPI anchor is made by plant cells and how it directs its attached proteins to the cell surface. Work already done in my laboratory has revealed much about the biochemistry of a GPI-anchored phosphatase capable of recruiting phosphate for the plant. We have already found a number of differences between the metabolism of plant and animal GPI anchors. The laboratory is now working with cultured *Arabidopsis* cells to learn how these modified proteins are formed and used.

9901798 Asymmetric Cytokinesis in *Pelvetia compressa*

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Strengthening Award; Grant 99-35304-8230; $100,000; 2 Years

Cell division is fundamental to growth and development in all organisms. However, the role of cell division in determining the shape (morphology) of animals and plants is very different. Animal cells are mobile and the shape of an animal depends on how cells arrange themselves. In contrast, plant cells are surrounded by a wall and cannot move. Morphology in walled organisms is determined by two main factors, the orientation of cell division and the direction of cell elongation. In this award, we will investigate the mechanisms that control cell division in plants. The experiments will be conducted using fertilized eggs of marine brown algae because we can get large numbers of these cells quickly and they undergo a precise pattern of cell divisions in early development. We will investigate two important questions: 1) How is the orientation of division chosen? and 2) What are the components of the cellular machinery that actually divides the cell? From this work, we hope to gain insight into how plants control their shape during development.

9901871 Phosphoinositide Signaling in Plants

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Strengthening Award; Grant 99-35304-8114; $100,000; 2 Years

Intracellular signaling molecules can regulate eukaryotic cell growth and stress-response. This research focuses on how these signaling molecules control plant cell growth and stress-response. Members of a class of intracellular plant signals called phosphoinositides have an inositol ring head group and two fatty acid tails. These molecules reside in cellular membranes and, we hypothesize that when the inositol head is modified via the addition of phosphate groups, specific phosphoinositides can bind to regulatory proteins and alter their cellular location and/or activity. In addition, a metabolite of one of the phosphoinositides apparently causes calcium levels to increase dramatically inside cells. This research will explore how phosphoinositides and phosphoinositide metabolites serve as signaling molecules in the simple plant, *Arabidopsis thaliana*. We will examine the quantity and molecular species (there are 7 types) of phosphoinositides found in plant tissues during normal growth and under stress conditions (e.g., high salt growth conditions and elevated growth temperatures). Phosphoinositide-binding proteins will be isolated and we will examine whether calcium levels change in cells with high concentrations of specific phosphoinositides. Altogether, these efforts will help us understand how plants utilize membrane phospholipids to control growth and their response to stress. This basic information could potentially be used to engineer plants that are more resistant to stress.

9901809 The Role of *SLEEPY1 (SLY1)* in GA Signal Transduction

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New Investigator Award; Grant 99-35304-8103; $120,000; 2 Years

The plant hormone GA stimulates critical processes in plant growth and development including: (i) stem elongation, (ii) flower initiation and fertility, and (iii) germination. This project will clone a gene needed for plants to respond to GA, *SLEEPY1* (*SLY1)*. While much is known about GA hormone biosynthesis, little is known about the

mechanisms of GA response, and the GA receptor has not been cloned. Historically, genes encoding hormone receptors have been identified genetically as hormone-insensitive mutants. Attempts to isolate GA-insensitive mutants have been confounded by the fact that they should be unable to germinate. Our novel strategies for the isolation of such GA-insensitive plants recovered ten recessive mutations in *sly1* in the model plant *Arabidopsis*. These are the first recessive GA-insensitive mutants to display the full spectrum of phenotypes seen in severe GA biosynthetic mutants including dwarfism, poor fertility, and failure to germinate. Based on these phenotypes, we hypothesize that *SLY*1 acts early to positively regulate GA response. In order to test this hypothesis we propose to (1) complete the cloning of *SLY1* based on map location, (2) determine its nucleotide sequence, (3) genetically determine the position of *SLY1* in the GA signal transduction pathway, and (4) analyze the *SLY1* gene expression pattern. The *SLY1* gene will provide an important tool for manipulating GA-regulated processes central to the agricultural issues of pre-harvest germination, stand establishment, lodging, and yield.

9901833 Kinetics of Phytochrome-Regulated Growth in *Arabidopsis*

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University of Wisconsin, Madison; Department of Botany; Madison, WI 53706
Grant 99-35304-7953; $210,000; 3 Years

Sunlight represents one of several environmental components that plants require to grow and reproduce normally and competitively. It is used by plants both as a source of energy and information. The informational qualities of sunlight that plants are able to assess and utilize for optimizing their development and maturation include light intensity, light direction, spectral quality, and daylength. All plants, including those important to agriculture, possess diverse families of light receptor molecules that are used to monitor sunlight information. This award permits research that seeks to understand the molecular basis for how one family of light receptors, the phytochromes, utilize light information to regulate plant development. To do this, we will use a model plant *(Arabidopsis thaliana)* that is perfectly suited for the physiological and molecular genetic approaches that are required for this research. We will study light-induced regulation of *Arabidopsis* growth using a computerized high-resolution measuring system recently developed in our laboratory. With this, we intend to dissect the mechanism of light-induced growth regulation by comparing the response kinetics of normal plants to others immediately available in this species that contain mutated forms of either the phytochromes or vital molecular components with which they interact. These measurements will help us to determine the chain of molecular steps necessary to cause a plant to grow in accordance to the light information that it perceives. The knowledge gained from this model study is directly applicable to all agronomically important crops and will therefore contribute to the improved management of sustainable agriculture.

PHOTOSYNTHESIS AND RESPIRATION

Panel Manager - Dr. Willem F. J. Vermaas, Arizona State University
Acting Program Director - Dr. Liang-Shiou Lin

Towards expanding our knowledge of fundamental aspects of energy capture and utilization by plants, research funded by this program extends from studies on solar energy absorption to analyses of crop productivity. Research supported includes studies on primary events of light absorption, energy transduction and utilization; structure, synthesis, turnover and interactions of components of the photosynthetic and respiratory systems; mechanisms of carbon dioxide absorption and fixation; metabolism and biochemistry of energy-rich compounds; and translocation and partitioning of photosynthates in the whole plant.

9901753 Alteration of Redox Properties in Bacterial Reaction Centers
Williams, J.C.; Allen, J.P.
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Grant 99-35306-7766; $240,000; 3 Years

Our overall goal is to understand at a molecular level how proteins modify the properties of molecules that undergo oxidation-reduction reactions, which are at the heart of photosynthetic energy conversion. The mechanism of water oxidation remains an unsolved question despite the fundamental importance of this process for plants. The oxidation of water requires a very high potential of approximately 1 V. In simpler bacterial systems the potential is lower, but we have manipulated the bacterial protein such that the oxidizing capacity has increased from 0.5 V to over 0.8 V. This allows us to use this system as a model for investigating the role of tyrosine amino acid residues, which form a link between water and photosystem II of plants. By substituting tyrosine residues in similar positions in the highly oxidizing bacterial reaction center, we have demonstrated that we can introduce a new electron transfer reaction involving the tyrosine residue. This capability provides a novel experimental basis for the determination of the factors that are critical for the participation of amino acid side chains in oxidation-reduction reactions. For example, the distance and the energy requirements can be tested as well as the coupling of protons to the electron transfer. Together these results should yield insight into the molecular properties needed for the utilization of highly oxidizing cofactors for electron and proton transfer in biological systems.

9901785 CO_2-Fixation and Metabolism in Green Plants: Support for Gordon Research Conference
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University of Arizona; Department of Biochemistry; Tucson, AZ 85721-0088
Grant 99-35306-7616; $10,000; 1 Year

This proposal asks for support of the September 1999 Gordon Research Conference on "CO_2-Fixation and Metabolism in Green Plants". The conference will be held in Oxford, UK, close to where one of the giants in our field, the recently tragically deceased Tom apRees, began to analyze plant metabolism, inspiring a generation of colleagues. Oxford is a place to pass on the baton to the next generation. We ask for the support of young US scientists to enable them to meet their peers in Europe, because the research field on carbon fixation and metabolism is much more active in Europe than in the US. The reason for this discrepancy is that the field is well-funded in Europe and has attracted much attention from industries that wish to modify starch and oil content of storage tissues.

9901748 Canopy Photosynthesis and Water Use Efficiency as Affected by Environmental Variables
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Grant 99-35306-7793; $160,000; 2 Years

Crop productivity, measured as dry matter gain, is mostly the result of net photosynthetic assimilation of carbon dioxide from the atmosphere by foliage canopy of the crop. Canopy photosynthesis is strongly influenced by air carbon dioxide concentration in the air and by climate and hence weather factors. In areas where crop production is limited by water, photosynthesis and water use are closely linked, showing a hand-in-hand relationship. With the anticipated global changes in climate associated with emission of carbon dioxide and other greenhouse gases, it is important to know, as quantitatively as possible, how productivity would change and how the change would be associated with changes in water use.

The project will investigate the validity and limitations of a set of simple equations developed earlier for predicting the relative or percentage changes in canopy photosynthesis and water use as weather and plant factors vary. Preliminary results indicate that the predictions made by the equations can be very good, but more thorough testing is needed. Experiments will be conducted in the open field, taking advantage of the natural short-term (5 or 10 minutes) variations in solar radiation, temperature, humidity, air carbon dioxide concentration, and wind, and the long-term (weeks and months)

variations in temperature and plant growth stages. If proven to be valid for most of the situations, the equations would provide a simple and yet powerful means for predicting crop productivity responses to the anticipated global changes.

9901759 Structure of Cyanobacterial Carotenoid Proteins

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Grant 99-35306-7908; $179,000; 3 Years

The carotenoids are a diverse group of pigments with a variety of functions in biological systems. They are found in all photosynthetic organisms, where they perform important roles in light-harvesting and photoprotection. In light-harvesting they capture wavelengths of light that other pigments cannot, thereby extending the spectrum of light that can be used in photosynthesis. In their photoprotective role they quench harmful molecular radicals that may be generated under excessive light. Carotenoids carry out these functions as components of pigment-protein complexes. The spectral and electronic properties that are critical to their function are "tuned" by their protein environment. We are studying two carotenoid-proteins from the cyanobacterium *Arthrospira maxima* : the orange carotenoid protein (OCP) and the red carotenoid protein (RCP). The RCP is a fragment of the OCP; removing amino acids from both ends of the OCP shifts the spectrum of the carotenoid and changes the color of the protein. Our goal is to determine the three-dimensional structure of the OCP and the RCP by X-ray crystallography. Comparison of the OCP and RCP structures will show, in atomic detail, the structural basis of their different spectral properties. Moreover, these structural studies will elucidate the organization of carotenoid pigments in this novel class of proteins, providing new insights into the pigment-protein and pigment-pigment interactions underlying energy capture and transfer. The information gained may be useful eventually in understanding and engineering light harvesting, energy transfer and photoprotection in higher plants or synthetic systems.

9901773 Direct Electrochemistry of Redox Cofactors in Photosynthetic Reaction Centers

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Grant 99-35306-7609; $160,000; 2 Years

Photosynthesis, the process by which light energy is converted into chemical potential by plants, algae, and some forms of bacteria, is the basis for all life on earth. The primary photochemical event catalyzed by the absorption of light by these organisms involves a special protein complex called the reaction center (RC) in which an electron is transferred by bound cofactors across a biological membrane, and the resulting electrical potential is stabilized. Despite years of intense research into the structure and function of the RC, a fundamental understanding is still lacking regarding the molecular factors that control electron transfer in photosynthetic systems. Understanding the mechanism of how electron transfer operates in the RC is central to several agricultural issues including plant resistance to stress, herbicide activity, and oxygen production. The goal of this research is to employ our novel synthetic approaches formulating thin RC protein films in conjunction with advanced voltammetric techniques to reveal the electrochemical properties of the RC. We will apply these techniques to RC proteins prepared from several photosynthetic organisms. These methods will allow, for the first time, direct monitoring of electrical events in the RC protein. Characteristic electrochemical properties of the proteins and their associated cofactors, and the effect structural modifications have on these properties will be obtained and are keys to understanding how the RC protein achieves efficient electron transport across biological membranes.

9901761 Structure and Function of Protein Disulfide Isomerase in Higher Plant Chloroplasts

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Strengthening Award; Grant 99-35306-8648; $100,000; 2 Years

This project focuses on the regulation of photosynthesis, the process by which plants produce oxygen and convert light energy into carbohydrate, protein and oils. Photosynthesis occurs on the photosynthetic apparatus located in subcellular structures, the chloroplasts. This apparatus is composed of the enzymes, factors and light-absorbing pigments needed for photosynthesis. The proper development of the photosynthetic apparatus is indispensable for crop productivity. High levels of sunlight and ultraviolet-B radiation (UV-B) reduce photosynthesis by damaging a protein, termed D1, in the core of the apparatus that is crucial for photosynthesis function. To maintain photosynthesis activity, plants have the ability to synthesize more D1 to replace the photo-damaged version. This "repair" process keeps the system operating. However, when the rate of photo-damage exceeds the rate of D1 synthesis, photosynthetic productivity is inhibited. This project uses molecular biological methods to determine the mechanisms that control D1 synthesis. We have recently isolated DNAs from genes for a key enzyme, protein disulfide isomerase (PDI), which is believed to regulate the rate of D1 synthesis - acting like a throttle - in response to light. This project will isolate the entire gene for PDI and the genes for other proteins needed for PDI function, and determining how these proteins function in the *Arabidopsis* plant. *Arabidopsis* is the most well-developed plant genetic system available. Research on this plant has yielded information applicable to crop plants. Isolating the PDI genes is a prerequisite which may allow us to manipulate photosynthetic productivity via biotechnology.

9901772 Structure and Function of Non-Symbiotic Plant Hemoglobins

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New Investigator Award; Grant 99-35306-7833; $210,000; 3 Years

We will conduct a study of the structure and function of a new, ubiquitous class of plant hemoglobins which are linked to the anaerobic response in plants and a possible new mechanism for respiration under low oxygen concentrations. Every year, these conditions are brought on by flooding which results in the loss of millions of dollars in agricultural products annually in the United States. The effects of flooding on crop yields can be ascribed to hypoxia resulting from the inability of oxygen to easily diffuse through water or waterlogged soil. The marked differences in survival under hypoxic conditions among different plant species suggest much room for improving the yields of most crops through breeding or recombinant techniques. However, we must first understand the biochemical factors which determine the ability of plants to survive hypoxic conditions.

This new class of hemoglobins (the nonsymbiotic hemoglobins or nsHbs) is distinct from the more familiar symbiotic plant hemoglobins involved in nitrogen fixation in some legumes. Because of their unusually large affinities for oxygen and low intracellular concentrations, it is unlikely that nsHbs are involved in oxygen transport. Instead, it has been suggested that they are involved in a new metabolic mechanism for energy production under low oxygen conditions which allows plant survival without the harmful buildup of fermentation products. The long term goal of our investigation of these proteins is to exploit their function for engineering the survival of crops in oxygen limiting environments.

9901784 Regulation of Triose Phosphate Export from Chloroplasts at Night

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Postdoctoral Fellowship; Grant 99-35306-8649; $90,000; 2 Years

Understanding the partitioning of photosynthetic carbon among starch, sucrose and other products is of fundamental importance to the production of crop plants with desirable agronomic and nutritive traits. However, the pathway and regulation of leaf starch degradation at night has received less attention than other components of the system. For many years, it was thought that the products of starch degradation were exported from chloroplasts at night as triose phosphates, phosphorylated three carbon sugars, which are the primary export product from chloroplasts during the day. Recent evidence has made it clear that neutral sugars are the primary export products of starch degradation at night rather than triose phosphates. However, it remains unclear why triose phosphates are not exported from chloroplasts at night. To explore this question, I propose to increase the rate of triose phosphate production at night in chloroplasts of wild-type *Arabidopsis thaliana* and an *Arabidopsis* mutant that lacks chloroplast neutral sugar transport. In the mutant, the change in levels of intermediate compounds and rates of starch degradation in response to increased production of triose phosphates in chloroplasts in the dark will allow me to test specific hypotheses about where the limitation to triose phosphate export at night occurs. The response of wild-type to increased triose phosphate production at night will provide insight into the evolution of independent mechanisms for carbon export from chloroplasts in the light and the dark.

9901750 Engineering the bc_1 Complex Family to Test Functional and Structural Differences

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Grants 99-35306-7009; $240,000; 3 Years

Evolution of the quinol oxidizing complexes of photosynthetic and respiratory chains diverged over the first billion years of the biosphere, to give three broadly recognizable families. In the Bacteria, two main branches are apparent. The firmicutes and cyanobacteria are distinct from the proteobacteria, and the descendants of these two branches in the eukaryote cell, the chloroplasts and mitochondria, show the patterns established in this early phase of evolution. Although the basic Q-cycle mechanism appears to be the same, the differences have given rise to distinctive structural and functional properties, which are of importance both from the physiological point of view and from a practical perspective. Recent advances in the structural biology of these proteins have provided the opportunity to explore the basis of the different properties. Distinctive features include different subunits for the c-type cytochrome, structural differences between the cytochrome b and iron sulfur protein components, substantial differences in the thermodynamic properties of the redox centers, and differential sensitivity of the quinone processing sites to inhibitors. This latter feature has allowed development of a range of topical fungicides acting at the mitochondrial level, which are relatively non-toxic to plants. We will explore these differential aspects of structure and function through molecular engineering of the bc_1 complex of *Rhodobacter sphaeroides,* to introduce features characteristic of the b_6f and firmicute complexes. We will identify residues at the Q-sites of the complex likely to be involved in inhibitor resistance, and change these to explore the molecular basis of resistance.

991769 Inorganic Biochemistry of Photosynthetic Oxygen Evolution

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Grant 99-35306-7613; $240,000; 3 Years

Photosystem II is the enzyme complex that absorbs light, produces oxygen and initiates a cascade of reactions that convert carbon dioxide into sugars. Owing to their great complexity, the reactions that produce oxygen are still a topic of active investigation. It is widely agreed that four manganese atoms form the heart of the reaction, but why oxygen evolution should also require calcium and chloride ions is not understood. This research project will utilize photosystem II preparations that are well-defined in terms of proteins that regulate access of ions to the site of water oxidation. In this way, the concentrations of calcium or chloride in their respective sites of action will be manipulated. Questions to be addressed are: (1) Is calcium close enough to manganese to serve as a binding site for chloride or water? (2) Do calcium and chloride bind to one another during oxygen evolution? (3) At which steps in the water oxidation reaction does calcium influence the electron transfer activity of manganese? (4) Can chloride affect manganese activity at two distinct binding sites? Answers to these questions will contribute to a better definition of the mechanism by which water is oxidized, and how this reaction is regulated by the structure of photosystem II. This knowledge may in turn prove useful in understanding why environmental factors, such as temperature, can destroy photosystem II activity, and, along with other data on photosynthetic reactions, help to define conditions that may enhance photosynthetic activity in a wide variety of plants.

9901757 TAKs: Thylakoid Membrane Kinases Associated with Light Energy Transduction

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Grant 99-35306-7909; $140,000; 2 Years

The phosphorylation of proteins within the eukaryotic photosynthetic membrane is thought to regulate a number of photosynthetic processes in land plants and algae. Both light quality and intensity influence protein kinase activity via the levels of reductants produced from the thylakoid electron transport chain. Protein kinases from eukaryotic organelles have not yet been purified or cloned. We report here on the protein isolation and molecular cloning of TAK1, an *Arabidopsis* membrane bound threonine kinase of the thylakoid membrane that phosphorylates the light harvesting complex proteins (LHCs). The kinase activity is enhanced by reductant, is associated with the photosynthetic reaction center II and the cytochrome b_6f complex, and itself is the target of phosphorylation. TAK1 is the target of light dependent phosphorylation by another thylakoid protein kinase. Candidates for this kinase activity include TAK2, which copurifies with TAK1. Both TAK1 and 2 have similarities to the to the TGFbI and II receptor families and we conclude that thylakoid protein phosphorylation may be regulated by a cascade of reductant controlled membrane bound protein kinases.

9901776 Oxygen Isotope Fractionation by the Cyanide-Resistant Oxidase in Plants

Siedow, J.N.; Gonzalez-Meler, M.A.; Umbach, A.L.
Duke University; Department of Botany, DCMB Group; Durham, NC 27708-1000
Grant 99-35306-7774; $120,000; 2 Years

Plants possess an energetically wasteful respiratory pathway in their mitochondria termed the "alternative" pathway. Large amounts of potential harvested crop yield may be lost through the operation of this pathway. Although the role of the alternative pathway in metabolism is not known, it is found in all plants, suggesting an important metabolic function. Knowing under what circumstances electrons derived from respiratory substrates are diverted from the main, energy-producing respiratory pathway onto the alternative pathway is crucial to discovering the alternative pathway's role. Studies of the distribution of electrons between the two respiratory pathways are possible through a technique based on differential use of the stable isotope oxygen-18 by the two oxidases that terminate the two pathways. The continued development and application of this technology will allow characterization of alternative pathway activity during respiration by intact plant tissues responding to two environmental stresses, low temperature and phosphorus deprivation. These studies will be carried out using plants having the "normal" alternative oxidase, the enzyme that comprises the alternative pathway, and transgenic plants expressing an altered form of the enzyme that cannot be inactivated by normal regulatory mechanisms. This research will improve our understanding of conditions under which the alternative pathway is active, which is essential for any future agricultural application of transgenic plants with modified levels of the alternative pathway.

9901767 Rubisco Phylogenetic Correction

Spreitzer, R.J.
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Grant 99-35306-4525; $120,000, 2 Years

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is key photosynthetic enzyme that captures carbon dioxide for the production of starch. Although this reaction serves as the only entry point for atmospheric carbon into the biosphere, oxygen competes with carbon dioxide at the same Rubisco active site, reducing net photosynthesis by as much as

50%. If the enzyme could be genetically engineered to increase carboxylation or decrease oxygenation, an increase in plant productivity would be achieved. However, it is difficult to engineer Rubisco in crop plants because the site is produced by a gene that resides within the chloroplast. In contrast chloroplast genetic techniques are well developed for the green alga *Chlamydomonas reinhardtii*, and the structure of the *Chlamydomonas* enzyme is quite similar to the crop-plant enzyme. In past genetic studies, regions of the Rubisco protein were identified that influence the carboxylase/oxygenase ratio. The present objective is to genetically engineer these regions so that they will be identical to the structure of crop plant Rubisco. Because crop plant Rubisco is better than the *Chlamydomonas* enzyme, it will be possible to identify those regions that account for differences in carboxylation efficiency. Once identified, these regions would be the appropriate targets for engineering further improvement in crop plant Rubisco.

9901756 Quinone Dependent Electron and Proton Transfers in Bacterial Reaction Centers

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Strengthening Award; Grant 99-35306-7792; $120,000; 2 Years

Plants and photosynthetic bacteria have similar proteins that efficiently transforms light into chemical energy. The bacterial photosynthetic reaction centers (RCs) are smaller than those from plants and have a known 3-dimensional structure. The proposed studies focus on the RC quinones. Many commercial herbicides bind to PSII at a site homologous to the one that will be studied here. RCs bind quinones in 2 locations. An electron is transferred from the primary (Q_A) to the secondary (Q_B) quinone in the last of the intra-RC electron transfers that are initiated by light. Protons are bound to the protein on this reaction adding to the stored energy. For the reaction from Q_A to Q_B to be favorable, the protein must change the chemistry of each quinone. Studies will highlight the role of the protein in determining the quinone chemistry and in controlling the electron transfer and proton uptake. (1) The electron transfer from Q_A to Q_B is limited by some change in the protein. RCs have recently been prepared where the reaction can go much faster because the conformational change is not needed. The fast reaction will be characterized and the reasons for the conformational change determined. (2) The protein must modify the quinone chemistry so the electron transfer is favorable. Different compounds will be tested in the Q_B site to see how the protein changes the properties of bound quinones. (3) The changes of the protein structure that are required for the reaction will also be studied by measurements at very low temperatures.

9901743 Gordon Research Conference on the Biochemical Aspects of Photosynthesis

Golbeck, J.H.
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Grant 99-35306-7543; $12,000; 1 Year

This proposal requests support for a Gordon Research Conference on "Biochemical Aspects of Photosynthesis" to be held at the New England College, New Hampshire from June 13 to 18, 1999. The conference Chair is Dr. John H. Golbeck and the Vice-Chair is Dr. Gary Brudvig. Advances in the interdisciplinary field of photosynthesis are reflected in the core talks at the 1999 Conference in sessions devoted to the biochemistry and biophysics of Type I (Photosystem I, green sulfur bacterial and heliobacterial) reaction centers, Type II (Photosystem II, purple bacterial) reaction centers, cytochrome complexes, and light-harvesting antenna. An emerging research area which is yielding impressive results is the biogenesis, assembly, and turnover of the photosynthetic apparatus. Two sessions are devoted to this topic, including one on the environment and assembly of the manganese cluster in photosystem II and the other to the development and turnover of Type I and Type II reaction center complexes. An entire session is devoted to new information gleaned from the now-complete gene sequences of the anaerobic green sulfur bacterium *Chorobium tepidum* and the purple non-sulfur bacterium *Rhodobacter capsulatus*. Lessons learned from the natural photosynthetic complexes along with advances in organic photochemistry have led to the synthesis of artificial photosystems, and the conference includes a session devoted to biomimetic systems and protein engineering. The 1999 conference ends with an outside perspective on lessons learned from the assembly of the iron-molybdenum cofactor in the nitrogenase enzyme.

9901781 Biosynthesis of Phycobilins

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Grant 99-35306-7827; $130,000; 2 Years

Phycobilins are the molecules that give red and bluegreen algae (seaweeds) their characteristic colors. Phycobilins are very important to these plants and to the environment because they are used to absorb the energy of sunlight which is converted to food energy by photosynthesis. On a global scale, much of the carbon dioxide that is converted to organic matter is transformed by these plants. Moreover, much of the world's sustainable agriculture is based on traditional farming methods that use seaweeds and related micro-algae as fertilizer to supply nitrogen and other nutrients to the soil to sustain the growth of major food crops such as rice. To better utilize these algae in energy efficient and environmentally sound agriculture, it is necessary to learn how they carry out their essential photosynthetic and metabolic functions. One important

factor will be to learn how these organisms manufacture the phycobilins that are essential to their photosynthetic metabolism. The goal of this research project is to learn how phycobilins are made and how the algae that contain them regulate phycobilin formation to optimize their use in the conversion of the energy of sunlight to food energy by photosynthesis.

NITROGEN FIXATION/NITROGEN METABOLISM

Panel Manager - Dr. Kathryn VandenBosch, Texas A & M University
Acting Program Director - Dr. Ann Lichens-Park

Grants in this program support research which will improve our understanding of how nitrogen is fixed biologically, metabolized and cycled by crop plants. These goals are of importance to sustainable plant production, since availability of nitrogen is a common limiting factor in plant growth, and because of costs associated with application of nitrogen fertilizers. Areas supported by the program include, but are not limited to: chemistry of biological nitrogen fixation; factors limiting biological nitrogen fixation; plant-microbe interactions involved in establishing and maintaining the biological-nitrogen fixation symbioses; and metabolism of nitrogenous compounds by higher plants.

9903698 Physical Changes Associated with Stress-Induced Decreases in Nodule O_2 Permeability
Denison, R.F.; Shackel, K.A.
University of California, Davis; Department of Agronomy and Range Science; Davis, CA 95616
Grant 99-35305-8646; $105,000; 2 Years

Legumes such as soybean or alfalfa need little or no nitrogen fertilizer, because their root nodules contain rhizobia (bacteria) that can fix (take up) atmospheric nitrogen. Nitrogen fixation is unusually sensitive to stress, such as leaf removal by sheep or hail, however. Active regulation of nodule gas permeability by the plant is essential, allowing enough oxygen for rhizobial respiration, while preventing oxygen damage to nitrogenase, a key enzyme. Nodule permeability may be controlled by changes in the dimensions of air-filled pathways through which oxygen diffuses into the nodule, perhaps due to pressure changes in adjacent cells. So far, there is not enough experimental evidence strongly to support or to disprove this hypothesis.

A single-cell pressure probe and a laser rangefinder will be used to look for predicted changes in nodule regions that appear to be responsible for permeability control, in response to treatments previously shown to trigger permeability changes. These treatments include changes in external oxygen (simulating flooded soils or a change in rhizobial respiration rate), substitution of argon for nitrogen (simulating "ineffective" rhizobia that fail to fix nitrogen), defoliation, and nitrate exposure.

Information from this study on the physical mechanisms of nodule permeability control will be used, in conjunction with follow-up studies on biochemical and molecular aspects, to develop crops (maybe eventually including non-legumes such as corn) that have optimum rates of nitrogen fixation even under stressful conditions.

9903497 *Sinorhizobium* Lipopolysaccharide Structures Essential for Symbiotic Infection
Forsberg, L.S.; Carlson, R.W.; Reuhs, B.L.
University of Georgia; Complex Carbohydrate Research Center; Athens, GA 30602-4712
Grant 99-35305-8143; $210,000; 3 Years

The legumes (e.g. beans, peas, alfalfa) are commercially important crop and forage plants that are able to flourish in poor or unfertilized soils. This is because the roots of these plants become infected with certain bacteria (the *Sinorhizobium* bacteria), which are capable of removing nitrogen from the atmosphere and delivering it to the plant in a biologically useful form, a process referred to as nitrogen-fixation. The infection of these plants is thus a natural, beneficial relationship (symbiosis), in which the *Sinorhizobium* provide nitrogen to the legume, which in turn provide nutrients needed by the bacterium. Clearly this phenomenon is of extreme agricultural and economic importance, since the legumes are nutritious, high-yield crops and since it reduces the need for expensive, nonrenewable fertilizers. However, the nature of this process is not well understood. Many legumes can only be infected by specific strains of *Sinorhizobium,* and will only grow in parts of the world where these bacteria thrive. In addition, many of the modern, genetically improved varieties of legume display a narrow range of tolerance for the different types of *Sinorhizobium,* thus restricting the utility of these plants. There is considerable scientific evidence that certain compounds (polysaccharides) on the surface of the bacteria are responsible, in part, for facilitating symbiosis and controlling the specificity of this process. Our research will directly examine the structure of these compounds in several prominent types of *Sinorhizobium* growing under different conditions. The resulting biochemical information will increase our understanding of symbiosis and its effective utilization.

9903692 Single-Crystal and Solution ENDOR Studies of Nitrogenase
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Grant 99-35305-8643; $195,000; 3 Years

The biological production of ammonia from nitrogen of the air is of critical importance in the agriculture of legumes as well as other crops. This reaction is achieved in certain microorganisms by the nitrogenase enzyme. However, the function of this enzyme continues to defy our understanding, much less any efforts at its modification, or at achieving a

useful chemical alternative. The site of ammonia formation is incorporated into a component of nitrogenase called the MoFe protein. We are studying this component with a kind of 'spectroscopic microscope' that can provide microscopic information about the composition, structure, and bonding of key intermediates in the chemical process. Our aim is to identify the atomic features that control the exquisitely tuned catalytic mechanism of nitrogenase, and thereby to help explain the underlying chemical events of ammonia production.

The techniques we employ combine nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) and are called electron-nuclear double resonance (endor) and electron spin-echo envelope modulation (eseem). They have already given the first microscopic insights into the properties of reaction intermediates, and in favorable cases, like nitrogenase, they offer the prospect of characterizing every atom involved in the reaction at a catalytic active site.

9903695 Mechanism of Nitrogen Fixation

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Grant 99-35305-8645; $240,000; 3 Years

Nitrogen fixation, the conversion of dinitrogen from the air into ammonia, is directly related to world-wide crop production and, as such, is one of the most important enzymatic reactions in nature. The nitrogen-fixing enzyme, nitrogenase, is synthesized in a relatively small number of diverse soil-living bacteria as well as photosynthetic bacteria and cyanobacteria. Of all the ammonia present in the world, well over 60% has been generated from bacterial nitrogen fixation. Obviously, it would be of great importance to agricultural yield to have a clear understanding of the mechanism of this system. However, in spite of the large volume of research that has been done on this enzyme, the mechanism of nitrogen fixation and the binding mode needed for substrate reduction is still unclear. The purpose of this research project is to gain a better understanding of this mechanism by using EPR and ENDOR spectroscopic techniques to study intermediate states of both normal and mutant forms of the Mo-nitrogenase enzyme. These techniques will be used to detect and elucidate the mode of binding as well as the binding sites of various substrates as well as the inhibitor CO. Kinetic studies will also be undertaken to allow a better understanding of the changes that occur in the nitrogenase enzyme during the conversion of substrate into product. The results of these experiments will provide very important information to further our understanding of this basic enzymatic process.

9903502 12th International Congress on Nitrogen Fixation

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Grant 99-35305-8405; $8,000; 1 Year

Nitrogen is one of the most important elements needed by crops for maximum yield and quality. Symbiotic nitrogen fixation by legume plants, such as alfalfa and soybean, plays a critical role in sustainable agriculture by providing 90 million metric tons of nitrogen each year. This process occurs in small wart-like structures called nodules on legume roots. Symbiotic bacteria inside the nodule convert atmospheric nitrogen gas into a form that the legume plant can use. Therefore, legumes need little if any nitrogen fertilizer. Moreover, legumes can provide significant nitrogen to other crops like wheat and corn when grown in rotations. By growing legumes farmers can reduce the amount of nitrogen fertilizer they need to purchase and apply. In addition, the nitrogen gained through symbiotic nitrogen fixation is less likely to contaminate ground- and surface water. Every two years an international conference is held which brings together scientists from throughout the world doing research on nitrogen fixation. This conference allows scientists from the United States to discuss and report on current scientific advances made in the science of nitrogen fixation. The 12th International Congress on Nitrogen Fixation will be held in Brazil. The $8,000 awarded by the USDA/NRI Program will be used to help pay in-country expenses for 12 U.S. scientists attending the Conference.

9903690 Alanine Excretion by Soybean Nodule Bacteroids: Role of Alanine Dehydrogenase

Emerich, D.W.
University of Missouri, Columbia; Department of Biochemistry; Columbia, MO 65211
Grant 99-35305-8144; $133,000; 2 Years

Symbiotic nitrogen fixation is the predominate process by which the inert pool of atmospheric nitrogen gas is converted into a form that can be used by living organisms. The symbiosis between soybean plant and the bacterium, *Bradyrhizobium japonicum* provides a useful model system for understanding how the nitrogen reduced by the symbiotic form of the bacterium is transferred to the host plant. Previous models have implied that ammonium, the first product of nitrogen reduction, was transferred to the plant by diffusion. However, recent evidence has demonstrated that the ammonium formed within the bacterium was first incorporated into the amino acid alanine before transfer to the plant. The advantage of alanine as the primary nitrogen transfer product is that it is rapid, immediate and more efficient than diffusion. This proposal will attempt to further document that alanine, and not ammonium, is the primary product transported to the

soybean plant. The strategy to demonstrate the role of alanine in nitrogen transfer is to isolate the gene required for the synthesis of alanine, which is called alanine dehydrogenase, inactivate it and create a mutant of *B. japoniacm* without alanine dehydrogenase activity. The hypothesis is that this mutant will not be able to effectively transfer the reduced nitrogen to the plant. The plants inoculated with the mutants should show the typical symptoms of nitrogen deficiency as well as retardation of growth and development. This work will be used to increase the nitrogen nutrition of leguminous crop plants.

9903496 Nitrogen Fixation in Photosynthetic Bacteria: New Gene Regulatory Mechanisms

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Grant 99-35305-8647; $240,000; 3 Years

A renewed interest in the process of nitrogen fixation has stemmed from the increased publicity of an important environmental concern: the application of artificially fixed nitrogen (fertilizer) to millions of acres of farmlands has directly led to profound, worldwide ecological problems. A long- term goal for agriculture must be the decreased dependence on chemically produced fertilizer and the increased use of natural, biologically fixed nitrogen in the soil. This goal will require a much better understanding of how bacteria fix nitrogen and how they control the nitrogen fixation process. For example, the genes required for nitrogen fixation *(nif)* in the model phototroph, *Rhodobacter capsulatus,* are expressed only when both oxygen and sources of fixed nitrogen are limiting. A major distinction between other nitrogen fixing bacteria and *R.capsulatus* concerns the nitrogen-regulated, NtrC-dependent activation of *nif* genes. In *R. capsulatus* the RcNtrC regulatory protein activates the major housekeeping sigma 70 /RNA polymerase rather than the alternative sigma 54 /RNA polymerase for nitrogen-dependent, transcriptional activation at a distance. Because the RcNtrC system represents a new type of bacterial regulatory system, this study encompasses not just nitrogen fixation research but gene regulation in general. Molecular mechanisms of RcNtrC-mediated gene control will be studied. Specifically, it will be determined in Aim 1 what subunit(s) within sigma 70 /RNA polymerase interacts with the RcNtrC activator; in Aim 2 the parameters necessary for RcNtrC to contact RNA polymerase at the *nifAI* promoter; in Aim 3 whether a possible direct link between photosynthesis and nitrogen control exists at the *nifA2* promoter. Aim 4 is to further characterize the novel RcNtrC-mediated translational control of *nifR3* expression.

9903686 Iron Metabolism in the Bradyrhizobium Japonicum/Soybean Symbiosis

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Grant 99-35305-8644; $240,000; 3 Years

The increasing world demand for protein and the increasing expense of producing nitrogen fertilizer has generated a widespread interest in biological nitrogen fixation. Although the nitrogen-fixing *Bradyrhizobium*/legume symbioses supply much of the fixed nitrogen for agriculture in the temperate regions of the world, many aspects of the symbioses are still poorly understood. The research proposed here is aimed at obtaining a better understanding of the role of iron in the symbioses, given the prominent role of iron-containing proteins such as nitrogenase and leghemoglobin in the nitrogen fixation process. Such an understanding should elucidate how rhizobia interact with their plant host to acquire enough iron to establish an effective symbiosis. Studies will be carried out on iron uptake in the *Bradyrhizobium japonicum/soybean* symbiosis. The experiments are all directed at answering a central question: How do bacteroids acquire iron in planta? Specifically, we want to know 1) are bacteria using ferric or ferrous forms of iron when they are in the nodule, 2) does the iron regulatory protein Fur control genes important for the symbiosis and 3) are there nodule-specific ferric chelate reductases and iron transporters that deliver iron to the bacteria when they are living in the nodule? Our lab has recently cloned the genes encoding one of the iron-regulated outer membrane proteins of *B. japoniucm,* the Fur protein from *B. japonicum,* as well as the Fe(III) chelate reductase and a presumptive Fe(II) transporter from Arabidopsis, so we are well positioned to now examine iron uptake in both symbiotic partners.

9903489, 5-Aminolevulinic Acid (ALA) Formation in Legume Root Nodules

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Grant 99-35305-8062; $160,000; 2 Years

Soybeans and other legumes are extremely important to agriculture in the United States and throughout the world. These plants are able to grow under conditions in which other crops such as corn and wheat cannot because legumes establish a relationship with beneficial bacteria which provide them the essential nutrient nitrogen in a usable form. This group of bacteria, collectively called Rhizobium, reside within nodules on the legume root, and converts nitrogen in the air into ammonia by a process called nitrogen fixation. In our view, nitrogen fixation must be a central feature of sustainable agriculture strategies. Thus, we must understand the molecular basis of these interactions toward the goals of maximizing the benefit to the legume, and extending the process to other important crops by genetic engineering.

Nitrogen fixation is energy-demanding for both the legume host and the rhizobia bacteria, and this project seeks to understand crucial aspects of the energy utilization system from the plant perspective in the soybean-*Bradyrhizobium japonicum* symbiosis. The compound *5-aminolevulinic acid* (ALA) is the basic building block of heme, a molecule necessary for respiration in all aerobic organisms. Whereas the amount of heme present in most plant tissues is low, it is extremely high in root nodules as part of a plant hemoglobin necessary for nitrogen fixation. Accordingly, ALA synthesis must increase dramatically during nodule formation, and we are working to understand how this induction occurs. We have identified a protein that controls an ALA synthesis gene, and have found that this protein is also regulated in a manner consistent with nodule development and function. Our primary objectives are to elucidate the function and regulation of this novel protein.

9903702 Role of Enod8 in Symbiotic Nitrogen-Fixing Nodule Development

Dickstein, R.
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Grant 99-35305-8574; $137,500; 2 Years

Symbiotic nitrogen-fixing root nodules are important because they provide a means for nitrogen to enter the food chain without the application of nitrogen fertilizer. A full understanding of the mechanisms controlling the development and function of root nodules will provide a knowledge base that should enable us to increase the efficiency of nitrogen fixation and possibly increase the number of plants able to form nodules with nitrogen-fixing bacteria. The work in this project focuses on a plant nitrogen-fixing root nodule-specific protein: Enod8. Previous work shows that the Enod8 gene is expressed relatively early in nodule development, indicates that Enod8 is involved in nodule organ formation, and predicts that Enod8 is an enzyme with the ability to cleave lipids and/or esters. Probing the biochemical functions of nodule-specific proteins is critical to understand their roles in the nitrogen-fixing symbiosis. Recent data from our lab show that Enod8 co-purifies and co-migrates with an enzyme that can cleave certain types of esters. The most important goal of this project is to complete the purification of Enod8, either from nodules or from a recombinant system expressing Enod8, and fully characterize Enod8's biochemical activity. Experiments to do this are planned. We will also complete studies to determine Enod8's subcellular localization. These experiments will be done with nodules from alfalfa, *Medicago sativa*, an important crop plant, and also with the molecular genetically tractable model legume, M *truncatula*. The results of these experiments are expected to illuminate the function and role of Enod8 in nitrogen fixation, and thus contribute to the sustainability of U.S. agriculture.

9903683 Bacteroid-Specific Gene Expression in *Bradyrhizobium japonicum*

Stacey, G.
University of Tennessee; Department of Microbiology; Knoxville, TN 37996-0845
Grant 99-35305-8323; $137,500; 2 Years

Bradyrhizobium japonicum is a bacterium that can infect and establish a nitrogen fixing symbiosis with soybean. This symbiosis is of great agricultural importance. The exchange of signals between host and symbiont is critically important for the development of the nodule organ in which the bacteria reside. Although additional signaling occurs during later stages of the symbiont-host interaction, there is little information concerning such events. It is at this stage that nitrogen fixation takes place and, therefore, these later events could have a major agricultural effect. We have identified two genes *(nfeC* and *hsfA)* in *B. japonicum* that are expressed in a bacteroid-specific manner (i.e., only within the bacteria inside the nodule). Until our work, virtually all of the known bacteroid-specific genes had been shown to be regulated by oxygen. However, neither *nfeC* or *hsfA* expression is regulated by oxygen. Interestingly, *hsfA is* transiently expressed in the nodule first appearing around 12 days and then no longer expressed by 28 days, even though the nodules are actively fixing nitrogen. This is quite an unusual expression pattern that suggests novel modes of regulation. The *hsfA* gene has a RpoN-like promoter suggesting the involvement of a regulatory protein. We will seek to further define the *hsfA* promoter and to identify the regulatory protein required for *hsfA* transcription. Our goal is to identify the unique chemical and/or environmental signal that leads to *hsfA* expression. This research will provide important information concerning the nodule environment and may lead to strategies to increase nitrogen fixation.

9903684 V-Dependent and Fe-only Dinitrogenases

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Grant 99-35305-8532; $72,000; 2 Years.

The conversion of inert dinitrogen gas to ammonia is called nitrogen fixation. This process has practical agronomic importance because the availability of fixed nitrogen - commonly referred to as 'fertilizer nitrogen' - usually limits crop production. Biological nitrogen fixation, catalyzed by the enzyme nitrogenase, only occurs in certain microorganisms and it provides the major source of nitrogen that is available to all other organisms. Most nitrogen-fixing organisms use a nitrogenase that has molybdenum at the active site and it has been presumed that molybdenum chemistry is intimately

associated with the chemical mechanism of biological nitrogen fixation. This project is concerned with the biochemical analysis of nitrogenases that use vanadium or iron instead of molybdenum. The specific role of molybdenum, vanadium, and iron in these various nitrogenases will be assessed and compared. The goal is to understand the chemical mechanism of nitrogen fixation and to use this knowledge to develop effective molybdenum-, vanadium-, or iron-based synthetic systems for ammonia production.

9903498 Functional Roles of Nitrogen-Responsive Lipoxygenases

Grimes, H.D.
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Grant 99-35305-8267; $210,000; 3 Years

Nitrogen is required for all life forms to exist. Plants play a critical role in the net positive input of nitrogen into life on this planet by assimilating this element from the soil. Plant growth, development, and productivity depend on the assimilation and utilization of nitrogen. These processes, in turn, impact human health by providing the essential nitrogen in our diet. Because nitrogen assimilation and utilization is directly linked to plant productivity and agricultural yield, it becomes important to understand the biochemical and genetic bases of these processes. Attempts to improve crop yield must ultimately depend on it. Our laboratory discovered that an enzyme family, termed the lipoxygenases, function in nitrogen metabolism at several levels. One specific lipoxygenase form serves as a biochemical site for the temporary storage of nitrogen in the leaves of soybean plants. As seeds develop, this lipoxygenase is utilized as a nitrogen source and thus becomes important in the overall partitioning of nitrogen into the harvestable seeds. We have also demonstrated that this storage lipoxygenase accumulates in defined cells in the leaf and, furthermore, in distinct vacuoles. A major objective of this grant is to examine the role of these functionally distinct vacuoles in nitrogen metabolism. Other nitrogen-responsive lipoxygenases are present in the cytosol of defined cells and we hypothesize that enzymatically active lipoxygenases may function to mobilize nutrients into the developing seed. Thus, another objective is to characterize the biochemical features of these nitrogen responsive lipoxygenases.

9903491 Analysis of the ADP-Ribosylation System of R. Rubrum

Roberts, G.P.
University of Wisconsin, Madison; Department of Bacteriology; Madison, WI 53706
Grant 99-35305-8010; $157,000; 2 Years

Nitrogen fixation is the process by which certain organisms convert abundant N_2 gas into a form of “fixed” nitrogen that can be used by virtually all organisms as the sole nitrogen source. Among the organisms that rely on this process are legumes, for which nitrogen is fixed by symbiotic bacteria, but many other plants gain some nitrogen from bacteria that exist in the vicinity of their roots. The process of nitrogen fixation is very energy-demanding and is therefore tightly regulated by organisms that are capable of it. This regulation makes sense for nitrogen-fixing organisms, but not necessarily for human agriculture. One process by which some organisms turn off nitrogen fixation under some circumstances is by addition of a specific chemical group (ADP-ribose) to a protein critical for nitrogen fixation that results in its inactivation. When conditions change, so that nitrogen fixation is again desired, the chemical group is removed, restoring nitrogen fixation. In the present grant period we will continue to study the regulation of the proteins that add and remove this ADP-ribose group by following up our observations that certain global regulatory proteins are likely to be involved in nitrogenase regulation. We will create a variety of defined mutations in the genes encoding the global regulatory factors and determine the precise nature of their effects on nitrogenase activity. The results will affect our understanding of both the global and local regulatory systems. An understanding of this process of the addition and removal of the chemical group could allow us to optimize the process so that nitrogen fixation is maximized, with a resulting decrease in the amount of necessary nitrogenous fertilizer.

MARKETS, TRADE, AND RURAL DEVELOPMENT

The purpose of this Division is two-fold. One is to support research to enhance the global marketability and competitiveness of U.S. agricultural, forest, and fish products. Research includes development and/or application of methodologies to assess product preference, demand, and utilization by consumers and factors affecting competitiveness including resource availability, costs, new technologies, market structure, and risk. Public policy research is supported that evaluates market development and competitiveness impacts as well as alternative fiscal and monetary policies, trade promotion, sustainable agriculture and communities, human resources, effects of research programs, and impacts of other programs and regulations.

The second purpose of the Division is to develop and/or apply research methodologies to problems affecting economic welfare and quality of living in rural communities and people by examining the effects of international, national, state and local policies. Research involves comparative analysis of the impacts of policy options and alternatives on natural resource use, population distribution, and the structure of rural society. The goal is to develop new economic development opportunities to sustain rural communities and people in the United States.

MARKETS AND TRADE

Panel Manager - Dr. Mary Marchant, University of Kentucky
Program Director - Dr. Mark R. Bailey

The goal of this program is to support research aimed at assessing market preferences, demand, and utilization for various agricultural and forest products and commodities; determining the ability of the U.S. to compete for these markets; and assessing the impacts of new products and production technologies on U.S. competitiveness, the environment, and rural economies. Research was specifically encouraged to: (1) identify, describe and quantify the size of potential international markets; (2) ascertain the ability of the U.S. to compete in particular agricultural global markets; (3) risk analysis and cost benefit analysis dealing with food safety and other risk management scenarios; and (4) determine the benefits and costs of adopting new products and/or production/processing methods for agricultural and forest materials produced using sustainable and alternative agricultural and forestry practices.

9901614 The Effect of Biotechnology Developments, Vertical Coordination, and Seed Market Concentration on U.S. Competitiveness in Agriculture

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Grant 99-35400-7869; $40,000; 1 Year

This project is designed to follow-up on a previous survey of biotechnology companies providing genetically modified seed stocks in order to estimate the distribution of welfare from the use of Bt cotton and herbicide-tolerant soybeans in 1997. During the year of this project, a survey instrument will be finalized to obtain data needed to compile the current annual financial and human resources that are devoted to plant breeding research and development activities by public and private sectors in the U.S. A comprehensive list of institutions, companies, and individuals employed in these endeavors will eventually be compiled, including mailing addresses. This project will also permit additional work to finalize the models to be employed in determining the magnitude of economic surplus, consumer surplus, and producer surplus resulting from the adaption of these technologies.

9901729 The Political Economy of U.S.-Canada Trade in Softwood Lumber and Newsprint

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Grant 99-35400-7741; $72,000; 1 Year, 6 Months

This project is meant to systematically assess the economic and political decision making in the U.S. Trade in softwood lumber and newsprint with Canada. Softwood lumber and newsprint are two of the largest trade commodities between the two countries. The U.S. and Canada have experienced four rounds of softwood lumber trade negotiations in the past 18 years. The 1996 U.S.-Canada Softwood Lumber Trade Agreement (SLTA) called for a tariff-regulated quota to restrict Canadian exports to the U.S. These two countries had another trade tariff dispute on newsprint some eight decades ago, which has resulted in free trade in newsprint since 1911. This study will estimate the economic impacts of SLTA, assess the relative incidence and importance of legislative events in the newsprint tariff battle, and determine legislator responsiveness to different interest groups. By contrasting the two trade disputes, this study explains how U.S. consumers won the newsprint tariff battle and U.S. producers won the softwood lumber tariff battle. This project will yield much-needed information on the efficiency and distributional consequences of the SLTA, including how much the trade policy has enhanced the competitiveness of U.S. lumber industry, and will shed some light on the political economy of U.S.- Canada forest products trade. The results of this project will facilitate informed debates among policy makers, interest groups, and academicians on U.S. forest products trade policy and are relevant especially as the SLTA will expire in less than three years and the search for a long-term solution is already underway.

9901592 Retail Contracting and Grower Prices in Fresh Fruit

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Grant 99-35400-7690; $29,000; 1 Year

Just as the broiler and hog sectors have seen the extensive development of contracts between processors and producers, the U.S. fresh fruit sector is now experiencing marked growth in the use of contracts between retailers and growers/shippers. Currently, 56% of all produce shippers use retail contracts for at least 10% of their sales. Increasing concentration in the retail sectors suggests that this trend is likely to continue. While contracts are cited as a means for reducing retailer costs and insuring supply, they also result in the loss of open market pricing mechanisms. Using a model for a principal-agent relationship, this study will develop a theoretical model of the contracting relationship between retailers (principals) and produce shippers (agents). This model is then used to develop an empirical model which will test

hypotheses on the attributes of shippers likely to participate in retail contracts. Data collected from a nationwide survey of produce shippers will be used to estimate this model. Finally, through simulations, the effect of retail contracting on grower prices will be analyzed. This analysis will provide new insights on the impact of retail contracting on pricing efficiency in the fresh fruit and vegetable sector. It will help identify the effect of these contracts on the prices received by growers, the likely future structure of the produce packing industry, and the implications for consumers with regard to produce prices and availability.

9901595 Enhancing U.S. Fruit Market Access Among Japanese Consumers and Channel Members

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Grant 99-35400-7703; $110,000; 3 Years

The recent failure of U.S. apple exporters to successfully market their product in Japan and a continuous decline in the Japanese import market share of U.S. oranges call for a serious examination of Japanese market access issues and a competitive assessment of U.S. fruit. Much attention has been devoted to formal barriers. However, overcoming informal barriers related to consumers and channel members is even more essential to sustain and increase U.S. exporters' share of the Japanese fruit market. Using the Fishbein attitude-behavioral intention model, we propose to assess and compare market access issues faced by U.S. fruit exporters and their major competitors. This will be done by focusing on informal trade barriers associated with consumers and channel members. A five-phase methodological process (focus groups/in-depth interviews, pretest, nationwide mail survey, data analysis, and strategy development) will be used. Four separate date sets (consumers x channel members and oranges x apples) will be collected. Multivariate statistical techniques and a structural equation modeling technique will be employed for data analysis. The outcome of the study will reveal the competitive position of U.S. fruit through an analysis of: (1) attitudinal barriers of consumers and channel members; (2) social and business normative barriers; (3) the extent to which attitude and social/business normative components predict behavioral intentions; (4) the role that consumers' and channel members' direct experience and perceived behavioral control as well as consumer socioeconomic and business characteristics play in the model; and (5) the effects of relative pricing in the model (price contingent intention model). Based on the research findings, push-pull marketing strategies will be developed for implementation by U.S. apple and orange exporters.

9901622 Market Impacts of Vertical Coordination: The Case of Perishable Commodities

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Grant 99-35400-7822; $100,000; 2 Years

The shift toward increased consumer demand for convenience foods and other value-added food products has been accompanied by an increase in coordination between retailers and other actors in the marketing chain. Coordination facilitates the incorporation of retailers' information regarding consumer preferences into the production process. Increased retail concentration is likely to further increase coordination. Closer links between retailers and other actors will affect competitiveness throughout the marketing chain. Shippers may consolidate in order to supply the larger volumes demanded by larger retailers. Producers of perishable commodities, who already face considerable price risk, may face even more due to "thin" markets and greater price volatility. In turn, increased volatility of grower prices may strengthen the dependency of producers on their marketing agents. Many of these forces are already at work in the strawberry market. We propose to examine the effects of increased vertical coordination by examining pre-commitment agreements between retailers and strawberry shippers. Along with broilers, fresh fruits and vegetables have been leading sectors in the agricultural industrialization process. The strawberry industry is a natural candidate for empirical analysis, due to the historical record of the use of these arrangements. By examining one of these early innovators, we hope to enhance understanding of the nature of the industrialization process and its effects on the long-term competitiveness of American agriculture. Our research objective is to develop and test a market-level model of shipper' decisions regarding pre-commitment agreements based on Carlton (1979). We will investigate effects of pre-commitment agreements on the average farmgate price and volatility, shipper and grower revenues, and industry structure. Our methodologies and findings may apply to other currently less-industrialized products. Our analysis will advance the empirical assessment and evaluation of the increasing coordination which characterizes the industrialization of agriculture.

9901594 Trade Blocks in the Western Hemisphere: A Gravity Model Analysis

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Grant 99-35400-7740; $40,000; 1Year

The goal of this research is to analyze the effects of regional trade blocks in the Western Hemisphere on U.S. agricultural trade, and to predict the effects of the proposed Free Trade Area of the Americas. In 1994, thirty-four countries in the Western Hemisphere pledged to implement a Free Trade Area of the Americas by 2005. Such a Free Trade Area is

expected to be a comprehensive trade pact that will rationalize and liberalize the trade policies of the participating countries. While the technical negotiations have moved forward, the political process in the U.S. has moved more slowly. A reason for this uncertain political environment is the controversy over the economic impacts of such regional trade agreement. To achieve the goals of this research proposal, we will specify and estimate bilateral trade models, known as the "gravity model". The model links bilateral trade relations to important variables such as the distance between trade partners, gross domestic products of the trading countries, population, and other cultural factors. Also, the model is able to account for policy and price effects as well as membership in different regional trading blocks. The estimation of the gravity model at the disaggregated level would provide us with the tools necessary to evaluate the conomic effects of the the regional free trade agreements on the competitiveness of U.S. commodities.

9901618 Crop Patterns and Water Use Under Free Trade

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Grant 99-35400-7730; $62,000; 1 Year

Allocating non-market resources in a socially equitable or economically efficient manner becomes especially difficult when the opening of world markets changes the value of commodities produced by these resources, when the factors that use these resources are mobile across political boundaries and when the resources in question have limited mobility. One goal of this project is to determine the marginal value of water in three regions of the U.S. using a profit function based on farmers' optimal selection of crops and inputs in each region. The regions are northwest Florida, the San Joaquin Valley in California and the high plains of Texas. Using the same technique to estimate these values makes them comparable as estimates of the value of water currently available are not. Also, these values will be the result of farmers' profit-maximizing behavior and will be functions of world commodity prices and local input prices. A second goal is to compare these values across regions. Since the shadow values of water are derivatives of the profit function, the theory of factor price equalization can be used to test the hypothesis that these values are equal across regions. Finally, by treating water as a quasi-fixed variable in the profit function with a specific adjustment mechanism over time, the effects of changes in world markets or policy changes on water use in the future can be determined in this model through the effect on input or output prices.

9901613 Competitiveness of Agricultural Credit Markets

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Grant 99-35400-7867; $37,000; 1 Year

Over the past twenty years the competitiveness of domestic agriculture has become increasingly important and is dependent on the structure of input markets. However, following the deregulation of the domestic credit system in the late 1970's and early 1980's, competition in the domestic credit market has declined. The proposed research examines whether the reduction in competition in agricultural credit markets has let to the emergence of market power, which could potentially increase the cost of credit to domestic agriculture. Such a scenario would lead to increased production costs and decreased competitiveness for domestic agriculture at the same time that increased international competition becomes more prevalent. The major hypotheses are whether (1) declining rural bank numbers have led to oligopolistic behavior in rural credit markets; (2) the consolidation in the banking sector has led to a shift in the rural/urban distribution of bank members or lending; (3) either trend has been partially or completely offset by increased efficiency in the banking sector; and (4) the shifts in competitiveness, rural/urban concentration, or efficiency have offset the implicit subsidy enjoyed by agricultural credit markets in unit banking states.

9901597 Assessing the Potential for U.S. Cotton Exports to China

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Grant 99-35400-7707; $110,000; 2 Years

China's rapid economic growth and gradual transition towards a market economy have brought about significant changes in production and consumption patterns and trade behavior in its food and fiber sectors. With increased income and improved market accessibility, China has reversed from a net cotton exporter to a net cotton importer in the last several years. These changes have important implications for U.S. agriculture because China has become the world's largest buyer of U.S. cotton. The proposed research would examine the impact of various domestic agricultural and trade policies in China on the competitiveness of U.S. cotton exports to China and assess the potential importance of the Chinese market to U.S. cotton producers and processors. Specifically the research has the following objectives: 1) to identify the factors influencing regional cotton production and estimate the regional cotton supply response; 2) to analyze total fiber consumption and evaluate demand responses for individual fibers and substitution effects among fibers and nonfiber inputs in China's textile mill sector with respect to price and nonprice effects.; 3) to assess the potential importance of the Chinese market for U.S. cotton producers in the next decade by simulating various domestic agricultural and trade policy scenarios.

These scenarios include: China's new cotton price policy (i.e., canceling government support prices; World Trade Organization (WTO) accession; the MFA reform, and other macro policies); 4) to assess the impact of relocation in China's textile industry and cotton production; 5) to construct a time series cross section database for Chinese regional cotton production, consumption, prices, cost data, and regional output and input prices at the mill level, and the value and quantity of China's textile and apparel export by the major importing countries. The research will construct a detailed regional cotton model to estimate the regional cotton supply response. A LA/AIDS model will be used to estimate the total domestic fiber demand and a model of linear logit model for dynamic input demand systems will be used to analyze input demand relationship in textile mill sector. A two-region (eastern and western China) model will be developed to analyze the impact of movement in cotton production and relocation in textile industry. The study team will collaborate with College of Economics and Trade at Nanjing Agricultural University to collect data and create data set, which will be used for this study and make available for other U.S. based projections, such as ERS projections, FAPRI projection, and IFPRI projections, and other organizations, as appropriate.

9901599 Waste Management and Regulation: Impact on U.S. Hog Production Location and Trade

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Grant 99-35400-7756; $74,000; 2 Years

The US hog industry is facing two major structural changes: the emergence of new large and efficient operations coordinated with the hog-processing industry, and mounting environmental regulation on waste. On world markets, major hog producers face similar environmental regulations. Large US hog operations and processors are associated with our new status of net exporter of hog products. The research objective is to analyze the impact of environmental and waste regulation of the hog industry, emphasizing the interface between farm size, cost of compliance, competitiveness and production location. Specific objectives include (i) Assess the impact of waste regulation stringency and management costs on the domestic competitiveness of major and new producing states, and by farm size. The assessment includes the prediction of production location across states and their contribution to aggregate US pork exports; (ii) Assess the international competitiveness of the US hog industry relative to world competitors. The research plan is as follows. We will econometrically estimate and use plant-location models to analyze the domestic determinants of interstate distribution of hog production in the US, based on cost of production, including 3 alternative measures of environmental stringency and compliance costs by farm size, as well as cost of feed, labor, transportation, distance to markets and land prices and taxes. Using panel data, we will econometrically estimate a Hecksher-Ohlin model to analyze the international competitiveness of US hog producers, as explained by factors and cost of production such as feed, labor, transportation, environmental factors (regulation stringency, and natural endowments).

9901591 Future of Indian Grain Supply and Demand: Implications for U.S. Grain Exports

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New Investigator Award; Grant 99-35400-7697; $105,000; 2 Years

With its large and growing population, the Indian market for U.S. grain exports is limited only by policies that restricts imports and by relatively low level of incomes. In recent years, however, the government of India has implemented economic reforms that have stimulated economic growth and begun the process of liberalizing agricultural markets. Such changes open the possibility of rapid growth in import demand. The purpose of this project is to develop a theoretically consistent framework incorporating the effects of urbanization on food demand, the growing importance of livestock production and the associated demand for feed grains, regional variations in grain production within India, and the influence of domestic and trade policy interventions on production, consumption and trade. The model that will be constructed to analyze the prospects for U.S. grain exports to this important market includes an acreage allocation model to measure regional grain production, separate food demand models for rural and urban households specified as dynamic AIDS models, a feed demand model, and a model of livestock production. These models are linked to the world market through world prices, export and imports. Data for estimating the parameters of the model will be collected from secondary sources as well as through household surveys carried out by collaborators in India. The model will be used to analyze several scenarios concerning the likely evolution of Indian grain imports in light of increased urbanization, the production potential for Indian producers, income growth and continued policy reforms.

9901586 A Market Test of Irradiated Food

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Grant 99-35400-7795; $112,000; 1 Year

Although food irradiation offers the ability to greatly increase the safety of the U.S. food supply, very little irradiated food appears on grocery shelves because of grocers' concerns about adverse effects on consumer relations resulting from negative advertising by consumer advocacy organizations. This project will assess the ability of retail food stores with nationwide visibility to sell irradiated food without experiencing adverse customer relations resulting from potential negative advertising conducted by organizations opposed to food irradiation. The project will select two test sites to sell irradiated food. Given the aggressive stance taken by Food & Water, Inc. regarding the sale of irradiated foods, it is anticipated they will begin a media campaign to dissuade persons from purchasing irradiated products as the test sites. To allow for reasoned decision-making by consumers at these sites, and a fair market test of irradiated food, if Food & Water, Inc., or other organizations, run anti-irradiated media messages, the project will run advertisements that present the opinions of agencies supporting food irradiation. The project will serve as a test market for irradiated food and gather important information about how consumers evaluate complex and controversial food safety technology.

9901603 Estimating the Nationwide Demand for Risk Reduction from Foodborne Pathogens

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Grant 99-35400-7642; $120,000; 3 Years

Food safety regulations such as Hazard Analysis and Critical Control Points (HACCP) requirements will impose additional costs on the meat processing industry and these cost will inevitably be passed on to consumers. The overall objective is to determine the value that consumers place on reducing the risk of illness from pathogenic foodborne bacteria. We will focus on the value of reduced risk from E. coli 0157:H7 and Salmonella in the consumption of ground beef. The risk of illness from these pathogens can be reduced either privately (by cooking the meat more thoroughly) or collectively (with irradiation treatment or improved sanitation and testing in production). We will examine how consumer value for risk reduction is affected by alternative collective risk reduction strategies and by consumer preferences for degree of doneness in cooking. Other factors that may affect the value of risk reduction are the severity of the risk, the magnitude of the risk reduction and "who" is at risk — children or adults. We will also examine whether people have general preferences for food safety rather than pathogen-specific preferences. We will elicit values for risk reduction using both hypothetical survey methods and experimental markets wherein consumers make choices between alternative "risky" meals using their own money to "purchase" lower leels of risk. Prior to conducting the value elicitation studies, we will use focus groups to examine how best to communicate risk information to the participants. The risk reduction values will be cross checked against sales data for irradiated and pasteurized ground beef sold in a collaborating retail store.

9901588 Designing Effective Environmental Labels for Forest Products

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Grant 99-35400-7851; $140,000; 2 Years

From a policy perspective, the eco-labeling of forest products can educate consumers about the environmental impacts of a forest products manufacture, leading to changes in purchasing decisions, and ultimately, to reductions in negative environmental impacts. From a business perspective, eco-labeling allows firms to be awarded for their environmentally responsible forest management practices. The wide spread use of eco-labels suggests that they are perceived as effectively altering consumer behavior. However, few studies identify the effectiveness of alternative eco-labeling programs and no research has been conducted on the optimal form of forest product labeling. The objectives in this research is to: 1) identify the types of environmental information that consumers will find useful when choosing among forest products; 2) understand how different ways of presenting the information affects consumers' ability to comprehend and utilize the information; 3) understand how different ways of presenting the information affects consumers' perception of products' environmental impacts; 4) understand how 1)-3) differ across different types of consumers. Through the use of various statistical techniques we will be able to identify not only the magnitude of each respondent's errors and the effect of format on calculation time and effort, but also to determine if these errors caused a choice inconsistent with personal preferences. Together these will provide an opportunity to estimate the policy effectiveness of various labeling policies and the welfare implications of different labeling strategies.

9901623 Vertical Coordination and Environmental Regulation in Agriculture
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Grant 99-35400-7797; $92,000; 2 Years

Many pressing environmental problems in agriculture occur in industries where production is vertically coordinated, e.g., water pollution from poultry production, water and odor pollution from hog production, worker safety problems from pesticide use on processing fruits and vegetables, and bacterial contamination of eggs, poultry, and beef. Environmental quality problems in these cases arise from the joint actions of growers and processors. This project will investigate efficient and equitable design of environmental policies under these conditions. First, we will develop a theoretical model of the joint production of agricultural output and environmental quality under vertical contracts in which the grower is the residual claimant for environmental damage (e.g., manure disposal). We will use the model to examine theoretically the performance of alternative environmental policies in terms of efficiency (overall cost) and equity (distribution of costs between growers and processors). We will study policies such as taxes and regulatory restrictions on processor-supplied inputs (e.g., nutrient composition of feed) or on grower supplied inputs (manure disposal). Second, we will investigate the performance of these alternative policies empirically using simulations based on parameters characteristic of the poultry industry. Third, we will expand the theoretical modeling to other situations, including those with multiple types of environmental damage (e.g., odor and waste from hog production) and those in which the processor is the residual claimant for environmental damage (bacterial contamination of food, pesticide residues on produce). The results of the project will improve understanding of the costs and benefits of alternative environmental policies in agriculture, thereby enhancing both competitiveness and sustainability.

9901611 Networked Innovation: The Case of the Agrobiotechnology Industry
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Grant 99-35400-7821; $170,000; 2 Years

Agrobiotechnology is already a fledging commercial sector but its prospect value is much larger. Despite the prominence of multinational firms, technical leadership has so far been contained within national borders. The U.S. has so far demonstrated an almost total dominance in agrobiotechnology, both in ownership of intellectual property rights and in the commercialization of products from agrobiotechnology. Major mergers and acquisitions have also been contained within national or regional boundaries, taking place mainly among European or U.S. firms. Hence, an hypothesis can be made that technical leadership in agrobiotechnology confers national competitiveness gains. The project seeks to identify why certain firms (countries) are dominant today and whether their dominance will continue. Agrobiotechnology firms continuously adapt their strategies to win innovation races. The outcome of these races yields dominant players, but also molds industry structure and technical outcomes. To understand the underlying motives of firm strategies in agrobiotechnology and the economic and institutional forces that condition them, we must study a complex and dynamic network. This network has been shaped by large number of inter-organizational linkages and it will continue to be shaped by similar relationships well into the future. This research models the evolution of the agrobiotechnology network using network analysis. We analyze current and future network structures, and accordingly industry structure, conduct, and performance. The hypotheses that network position and membership in strategic groups of agrobiotechnology firms influence their strategic choices, inter-organizational linkages, and ultimately their performance, are tested.

9901630 The Impact of 2-Tier Tariff-Rate Import Quotas on Trade: An Assessment of the Agreement on Agriculture
de Gorter, H.
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Grant 99-35400-7729; $23,000; 1 Year

The research seeks to evaluate the effects on international trade of introducing 2-tier tariff-rate import quotas (TRQs) in agriculture. The primary purpose of TRQs was to increase market access for protected agricultural commodities through the quotas while tariffs were to maintain support to farmers. Although import quotas are binding (they cannot decline) and all tariffs (except some 1st tier tariffs) cannot increase, several issues have arisen in regards to the administration of TRQs and the methods of allocating the rights to import and export. There is no uniformity across countries in the setting of either the absolute or liberalization effects and unequal rents generated across countries. We identify the conditions under which either tier tariff or the quota is effective and describe their interaction as to their trade liberalization effects. A key problem has been the methods by which countries have been allocated quotas (or not) and the procedures (or lack thereof) for the distribution of the 'rights to rents' (for export and/or import licenses). We describe how imperfect competition and non-tradability of quotas and licenses will affect trade and economic inefficiency. Reasons for why import quotas remain unfilled include levels of 1st tier tariffs, and the allocation method of import trading firms. The extent of inefficiencies due to rent seeking is highlighted, along with how endogenous domestic policies allow governments to protect against the effects of trade liberalization and how secular changes in world prices affects TRQs. A modest

empirical research agenda is outlined to provide guidance for reforms in the upcoming 1999 negotiations in the Agreement on Agriculture.

9901637 Dynamic Behavior of Commodity Prices: Implications for Price Risk Management

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Grant 99-35400-7796; $33,000; 1 Year 6 Months

With the deregulation of commodity markets, price variability has increased. This implies increased price risk. Thus, individual firms' cost of managing risk have probably increased. The general objective of this project is to improve the conceptual and empirical models of agricultural commodity markets and to use them to appraise risk management strategies. If successful, the research will improve knowledge about price behavior in commodity markets and provide information for managing price risk. Specifically, the research aims to construct dynamic models of the corn, soybean, and milk markets, to obtain conditional probability distributions of monthly cash and futures prices, and to derive implications (from the empirical results) for marketing and hedging strategies of farmers. The research will involve five major tasks. First,a basic framework will be constructed, which is capable of reproducing the observed features of cash prices (autocorrelation combined with occasional large shocks). Second, the framework will be extended to accomodate the specific features of the corn, soybean, and milk markets. Third, the models will be solved and tested against market data, using simulation and estimation methods. Fourth, commodity futures prices for existing contracts will be generated from respective market models and compared to observed futures prices. Finally, the resulting empirical price distributions will be used to evaluate marketing strategies, especially from the viewpoint of evaluating their risk-reducing performance.

9901232 Devolution and Local Capacity to Implement Welfare Reform

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Grant 99-35400-7870; $80,000; 1 Year

This study investigates how poor rural counties implement new federal and state mandates of welfare reform under a system that devolves responsibility to local jurisdictions. We examine how human service agencies in counties located in rural, Appalachia Ohio differ in their approaches to determine what works and what obstacles prevent successful implementation. Agency policies, personnel, and clients will be surveyed in four counties that represent the region.

9901631 Technological Change and Dynamic Comparative Advantage of U.S. Agriculture

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Grant 99-35400-7696; $80,000; 2 Years

The United States remains the single largest agricultural exporter, accounting for an average of 18 percent of global agricultural exports during 1962-1994. Despite the continued growth in agricultural exports, the U.S. share of global agricultural exports has fallen by 8 percentage points since 1962. Much of the decline in the U.S. share is attributed to structural change in global agricultural trade, where processed foods are becoming increasingly important. With expectations for slow growth of domestic food demand, the continued success of U.S. agricultural and food sectors depends on foreign market opportunities, and so, the decline in U.S. share of world markets is of concern to policymakers. The basic hypothesis of the proposal is that productivity growth and technological linkages between the agricultural and food processing sectors explain export patterns and performance in U.S. and OECD countries' agricultural trade. Productivity growth which occurs due to the accumulation of knowledge can lead to not only efficiency in producing existing products, but also to the creation of new products and processes. Since bulk products constitute a major share of U.S. agricultural exports, there is an opportunity for U.S. agriculture to add value and reverse its decline in export markets. Empirical confirmation of the proposed hypothesis has important policy implications. Encouraging productivity growth and the transfer of comparative advantage from agriculture to food processing will help reverse the decline in U.S. export shares and lead to an increased share of processed commodities in U.S. agricultural exports.

9901626 Supply Chain Management for Commodities in Volatile Markets

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Grant 99-35400-7698; $100,000; 3 Years

The project will assess potential for enhanced supply chain management of procurer cost and supplier income risk through coordination across supplier pools and the procurer. Improved management of supply chain market risk will expand industrial demand for commodities, enhancing and stabilizing prices and farm producer/supplier incomes in the long-term. The project will design and assess multiperiod, financial implications for suppliers and procurers of central market and over-the-counter financial contracts and derivatives. Supporting objectives are to: 1) quantify price volatility for agricultural

commodities relative to key synthetic substitutes, and 2) assess benefits of risk management strategies for income across supplier pools and for procurement cost of commodity feed stocks. The approach will use stochastic dynamic mathematical programming models of the design and performance of independent supplier and procurer, supplier pools, and coordinated supplier-procurer strategies. Design will consider both pricing and risk sharing not possible using only market instruments. Models will be developed for supplier and procurers that face risk in commodity availability and levels, and prices. Heterogeneity across suppliers will be explicitly incorporated to allow evaluation of distributional implications and to understand the economics of pooling. Risk management strategies evaluated will include independent and pooled contract strategies using forward contracting, futures hedging, and options purchases. An application across the raw milk-dairy chain will assess feasibility of the approaches across a continuum of markets with differing risk management instruments and experience.

9901601 Relaxing the Jones Act: Cost and Dependability of Moving Grain to the Southeast

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Grant 99-35400-7704; $100,000; 2 Years

The Jones Act (Section 27 of the Merchant Marine Act of 1920) requires that all domestic maritime transportation be on vessels built, owned, operated, crewed and registered by United States Citizens. While vessels exist which meet the Jones Act restrictions, no deepwater vessels are used to ship grain between domestic ports. Thus, large-scale demanders of domestic grain with access to ocean ports such as livestock producers in the Southeast, must rely on unit trains to supplement local corn production. During delivery disruption emergencies, grain demanders have imported corn from Canada and other countries because no maritime means were available to deliver U.S. grain. The purpose of this study is to determine the impact of relaxing the Jones Act to allow use of non-U.S. vessels for domestic maritime transportation on the cost and dependability of transporting grain for animal feeding in the Southeast, especially for pork and poultry production in North Carolina. Procedures: 1) the POLYSYS regional corn acreage and yield projections will be used to estimate regional supplies. Regional demands will be estimated based on POLYSYS output and on regional information on per capita food demands, livestock production and rations, and local demand for industrial uses. Regional excess supplies (demands)

9901633 The Possibility of Private Crop Insurance and Reinsurance Markets

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New Investigator Award; Grant 99-35400-7755; $34,000; 1 Year

Because of the risk in agricultural production has prevented farmers from engaging in practices that are either more productive, profitable, or sustainable, effective risk management instruments, such as crop insurance, can help to improve productivity, profitability, and sustainability. The Federal government has been facilitating and subsidizing crop insurance to farmers for decades, which has cost tax payers millions of dollars and also provided disincentives to private insurers. An ideal crop insurance that can free the government resources would be one provided by private markets. This proposed research project is devoted to the investigation of the possibilities of such insurance/reinsurance markets both theoretically and empirically. It is believed that high correlations among farm yields have prevented private insurers effectively pooling the risk and providing affordable contracts. This belief is based on the lack of yield independency. A condition which allows for dependency, asymptotic nonpositive correlated distribution, has been found to facilitate risk pooling. The objectives of this project include applying the statistical theory in the content of crop insurance, testing the a.n.c. hypothesis on yields of major crops in the U.S. and /or other countries to support the idea of private insurance and reinsurance markets, and estimating the necessary scale of the insurance pool required by private insurers.

9901723 Modeling U.S., EU, and Canadian Dairy Policies Under Imperfect Competition

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University of Wisconsin; Department of Agricultural and Applied Economics; Madison, WI 53706-1513
Grant 99-35400-7868; $70,000; 2 Years

Dairy, one of the most heavily protected agricultural sectors in the EU, Canada and the U.S., will be a key focus of the 1999 WTO deliberations. Regional impacts (e.g., intra EU-15) and the interface of domestic policies with world markets (e.g., Canadian Class V, two-tiered export pricing with exports above GATT commitments) will be crucial to the political economy driving these discussions. As well, imperfectly competitive market structures in regional milk procurement and processing are increasingly important dimensions in the countries studied. This research provides a detailed policy/modeling tool to provide quantitative assessment of the changes in regional comparative advantage among U.S., EU, and Canadian dairy sectors in response to potential changes in international trade policies and regulations. This will enhance U.S. ability to retain domestic dairy markets and foster export growth by helping to better understand the forces that affect U.S. competitiveness

9901403 A Multi-County Analysis of Household Food Demand: Implications for U.S. Food Exports

Gould, B.W.
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Grant 99-35400-6112; $140,000; 2 Years

Understanding how food consumption responds to changes in relative prices, income, household composition and other exogenous factors is important for U.S. farm operators, processors, policy analysts and policy makers looking to expand the markets for U.S. farm products. This can be said not only with respect to domestic but also foreign markets. Once source of data useful for analyzing the structure of food demand is household-level surveys of food consumption and purchases. Use of household-level data for demand analysis however requires the applied researcher to account for the censoring (non-purchase) of commodity purchases. Ignoring the censoring of food purchases could potentially result in biased estimates of how food demand changes when prices, incomes, or other household characteristics change. When analyzing food demand one commodity at a time, there are a number of fairly standard econometric techniques that can be used to address this censoring. The statistical issues become much more complex when expanding the analysis from a single equation approach to a demand system framework. This project has as its primary objective the undertaking of some basic methodology development that will allow future analysts the ability to analyze the structure of food demand for specific commodities. Output from this project will be not only the development of new statistical methods for estimating food demand systems but also the empirical applicaion of these methods to food consumption data from a number of developing and developed countries.

RURAL DEVELOPMENT

Panel Manager - Dr. John K. Thomas, Texas A & M University
Program Director - Dr. Mark R. Bailey

Rural areas dependent on agriculture, forestry, and other natural resource based industries have been subjected to various forces that reduce their economic vitality. This program calls for theoretical and empirical research to help understand the ways these forces affect economic vitality and evaluate how well public policies might restore this vitality. Research is supported in three general areas: (1) new theoretical and methodological studies to focus on improving the social and economic well-being of rural families and communities at the national, regional and local levels; (2) empirical studies to identify the forces that influence population changes, employment, wage levels and other indicators of social and economic viability; and (3) empirical evaluations of methods and policies to diversify the economies of rural areas, particularly those highly dependent on agriculture, forestry and other natural resource extractive industries.

9901252 Impacts of State-Imposed Growth Management on Rural Areas
Reynolds, J.E.; Smith, M.T.; Steiner, R.L.
University of Florida; Department of Food and Resource Economics; Gainesville, FL 32611
Grant 99-35401-7728; $95,000; 2 Years

This project examines the effects of growth management on three types of rural areas in Florida: deep rural, exurban fringe, and exurban amenity. Florida is a bellwether state to examine the effect on agriculture and rural communities in the presence of a statewide growth policy, yet the study has broad implications as Florida is a prototype for what may happen elsewhere in the country. The study is designed to examine existing data and to conduct field work to assess the impact of growth management on Florida's rural areas. The research is timely because of the re-emergence of the topic of suburban sprawl and its impacts on rural areas throughout the country, as well as the expanding implementation of growth management in states across the country. Yet there has been little empirical research examining these impacts. Furthermore, the plans completed under the Florida Growth Management Act have been in place since 1988 and revisions to those plans will be completed during the duration of this grant, resulting in a ten year time frame for empirical analysis. Florida's diversity provides a good case study location, and the research includes explicit consideration of the types of rural areas in the state. The research will provide an extensive analysis of the institutional and fiscal capacity and other implementation issues of rural places in a diversity of settings as well as an analysis of land value changes resulting from the implementation of growth management.

9901224 Influence of Immigration of Older Persons on Rural Idaho Communities
Junk, V.W.; Carlson, J.
University of Idaho; School of Family and Consumer Sciences; Moscow, ID 83844
Grant 99-35401-7776; $95,000; 2 Years

The growing proportion of mobile older persons in the population has led many small communities to consider if they want to attract older persons to move to their community. With them, they bring both their resources and their eventual need for services. Those who have lived in the community for some time may either welcome the residents and help them feel part of the community, or resist the inflow of older persons. This project is designed to help small towns and communities make informed decisions and strategies regarding a potential influx of older persons. It will also help in identifying how older persons develop a sense of place when they move to a new community, such as through volunteering and joining local organizations. This will be accomplished by first meeting with focus groups of older immigrants, long-term residents, and businesspeople in three communities. The groups will discuss both the economic and social impacts of older persons on the community, and the impact of the community on new older residents. The selected communities are those which attract a higher than average proportion of persons age 65 and older. A telephone survey will be used, based on information gained in the focus group discussions.

9901241 Local Housing Decisions and Economic Vitality of Rural Communities
Cook, C.C.; Crull, S.R.
Iowa State University; Department of Human Development and Family Studies; Ames, IA 50011
Grant 99-35401-7769; $160,000; 3 Years

Although a sizable number of local housing decisions are made each year in rural communities, we do not understand the way the affect, nor their contribution to, rural vitality. The lack of such knowledge is an important problem, because without such information we cannot delineate coherent development strategies. There are two research objectives: (1) to identify the kinds of local housing decisions and activities that have occurred in rural communities in counties with high and low vitality; (2) to analyze the effect of local housing decisions and activities on the vitality of rural communities. Our hypothesis is that local housing decisions and activities do affect the vitality of rural communities by promoting or

failing to promote strategies that improve local housing situations for current residents and that draw potential new workers and retirees. The results of the study will increase our knowledge of the housing delivery process, one of the important forces influencing rural development. By identifying how and the extent to which housing affects rural vitality, our research findings will impact public policy and the vitality of rural communities in the future.

9901254 The Geography of Rural Financial Intermediation

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Grant 99-35401-7700; $165,000; 2 Years

Community banks are critical to rural development not only because they finance rural businesses and home mortgages, and manage savings, but also because of their voluntary and philanthropic activity in support of their communities. Banking regulations and banking technologies are also now changing radically. This project will document rural commercial banking to help identify what practices and bank structures are sustainable for both banks and rural communities. Also, it will provide a benchmark for future studies on the impacts of the regulatory and technical changes. It is an integrated research/action project coordinated by the North Central Regional Center for Rural Development with researchers at Iowa State University and community members in the Wallace Foundation for Rural Research and Development. The research goals are to (1) determine where rural savings go and from where rural investment comes, (2) determine if/how rural credit market participants (savers, borrowers, and community banks) are disadvantaged relative to their urban counterparts, (3) explain the observed patterns, and (4) relate the patterns in financial intermediation to rural development outcomes. The goals of this project are to inform community members, rural bankers, and policy makers; to form rural community investment working groups to design and implement new approaches to mitigate the costs of rural remoteness (on Rural banks as well as citizens) in capital markets; and prepare for the Internet age and the new "universal banking" regulatory environment.

9901234 Social and Economic Influences on Rural Small Business Competitive Strategy

Miller, N.J.
Iowa State University; Department of Textiles and Clothing; Ames, IA 50011

New Investigator Award; Grant 99-35401-7767; $120,000; 2 Years

Recognizing the important role small businesses play in strengthening the economies of rural communities, this project focuses on the strategies used by rural small business owners /managers to enhance their competitiveness in regional, national, and global markets. The net benefits of small business success are distributed community-wide. In addition to the provision of goods, services, and employment opportunities, small businesses contribute leadership and support to a broad array of community betterment efforts. The theoretical framework suggests that the social characteristics of communities can affect the success of small businesses. The research proposed here will examine the reciprocal and multifaceted relationship between small businesses and rural communities, specifically focusing on the embeddedness of small businesses in their community, their strategies for success, and their perceived and financial success. Features of the community, business, and the personal characteristics of the owner/ manager will also be examined. This investigation provides a new direction by measuring influences on the competitive strategies of rural small businesses and the relationships of these elements to business success. The unit of analysis will be small business owner/managers operating in two samples of Component Economic Areas (CEA), a concept developed by the Department of Commerce. These are multi-county units characterized by internal economic linkages. Small businesses in rural counties in CEAs with a metropolis and those in rural counties in non-metropolitan CEAs will be compared. This project will address a weakness in the literature, as well as provide insight into small businesses as a critical area of rural community development.

9901244 Developing Community-based Geospatial Technology Tools for Farmland Preservation

Tulloch, D.; Lathrop, R.; Parks, P.
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Grant 99-35401-7705; $145,000; 2 Years

As a counterbalance to the rapid loss of productive agricultural areas, farmland preservation programs have been established around the nation. However, the parcels set aside are often selected in a piece-meal manner, resulting in an inefficient and unsustainable agricultural landscape. Considering the significant investment made in farmland preservation, it is disturbing to realize that these investments are made without full consideration of whether the parcels will contribute to a larger, long-term, working agricultural landscape. The assessment of farmland for the inclusion in farmland preservation programs requires an evaluation of a host of environmental, locational, and socioeconomic factors. With the use of geospatial technologies (particularly GIS and remote sensing) the evaluation of potential parcels can be made in such a manner that the contiguous nature of some areas is considered. The evaluative process can also be aided by these technologies in applying

econometric models to determine parcels more likely to be developed and their appropriateness for long-term farmland usage. New Internet-based tools also allow members of the community to become more involved in the decision making process. We will develop and test appropriate geospatial tools and evaluative techniques that are applicable to a broad variety of farmland preservation contexts, policies, and programs. This will include the simulation of different policy outcomes and comparison of the impacts of each.

9901232 Devolution and Local Capacity to Implement Welfare Reform

Tickamyer, A.R.; Henderson, D.A.; White, J.A.
Ohio University; Department of Sociology and Anthropology; Athens, OH 45701
Grant 99-35401-7870; $80,000; 1 Year

This study investigates how poor rural counties implement new federal and state mandates of welfare reform under a system that devolves responsibility to local jurisdictions. We examine how human service agencies in counties located in rural, Appalachia Ohio differ in their approaches to determine what works and what obstacles prevent successful implementation. Agency policies, personnel, and clients will be surveyed in four counties that represent the region.

9901250 Environmental, Economic, and Community Impacts of Dairy Farms

Hanson, G.D.; Luloff, A.E.; Muller, C.D.; Hatley, O.E.
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Grant 99-35401-7874; $175,000; 2 Years, 6 Months

Traditional, confinement, and grazing dairy systems in Southeast Pennsylvania will be analyzed with reference to (1) community, (2) environment, and (3) economic sustainability. Nearly half (44%) of Pennsylvania's $1.6 billion dairy industry (4th in the U.S.) is located in this area. While confinement technology offers specialization and scale advantages, intensive grazing systems may be the most feasible for small dairy farms. Key informant survey methods will be used to identify important issues at the agriculture/community interface. Issues identified by key informants will then be surveyed in linked samples of farm and nonfarm households. Environmental and economic analyses will be conducted with APEX (Agricultural Policy/Environmental eXtender) and *FINLRB* (Financial Long Range Budgeting) on 30 baseline farms. Community well-being measures used in previous research on agricultural problems at the rural/urban interface will then be modeled to maximize community and farm well-being. There are three integrated simulation steps for the baseline farms: (1) maximize farm profitability, (2) maximize farm profitability subject to recommended U. S. Department of Agriculture conservation guidelines, and (3) maximize farm profitability subject to both conservation compliance and community well-being constraints. Production system, nutrient loss, and economic and community factors affecting dairy sustainability will be statistically measured using logistic regression and cluster analyses. The study will provide a comprehensive, integrated analysis of these three dairy systems in this region.

9901240 Women on U.S. Farms

Sachs, C.E.; Willits, F.K.; Findies, J.L.
Pennsylvania State University; Department of Agricultural Economics and Rural Sociology; University Park, PA 16802
Grant 99-35401-7699; $130,000; 2 Years

Farm women in the United States make significant contributions to their farms and farm households. They support the farm through engaging in farm tasks, making farm decisions, and working the farm organizations. In addition, many farm women support the farm through their work off the farm. These activities contribute to the profitability and maintenance of U.S. farms and farm households. Despite this, our current knowledge of women's roles in helping to support agriculture is limited. The purpose of the study is to expand the knowledge base concerning the status of women in agriculture and rural development. We will conduct a major survey of over 3,000 U.S. farm households. The survey is designed to allow comparisons to the last major survey of U.S. farm women conducted by Rosenfeld 20 years ago.

9901259 Socioeconomic and Environmental Impacts of the North American Free Trade Agreement on the Rio Grande Valley of Texas

Angirasa, A.K.
Southwest Texas State University; Department of Agriculture; San Marcos, TX 78666-4616
Grant 99-35401-7775; $60,000; 3 Years

The goal of this study is to investigate and analyze the economic impacts of NAFTA on the agriculture sector in the Rio Grande Valley of Texas, a vast agricultural area on the Texas-Mexico border. Our proposed study is important because (1) the region is the major gateway into Mexico, (2) agriculture is a major component of the region's economy, and (3) the agriculture sector of the Valley faces direct competition from the same or similar activities across the border. To achieve the stated goal, we will analyze and compare the pre-NAFTA period (1989-1993) with the post-NAFTA period (1994-1998) with respect to economic performance of the agriculture sector. We will use county level data on land use, acreage, yield, production, revenues, etc. to evaluate the economic impact of NAFTA at the county and regional levels. We will apply

profitability, efficiency, and solvency performance measures to evaluate the financial performance of different county and size farm operations. This analysis will allow us to evaluate farm performance over time and changes in that performance from pre-NAFTA to post-NAFTA periods. This micro-level study of NAFTA will help not only the policy makers at the federal, state, and local levels, but the local farm and community organizations as well as in addressing the positive and negative impacts of NAFTA, and in making a case “for or against” NAFTA’s expansion to include Latin America.

9901227 Rural economic Development and the Opportunity-Threat of High Level Nuclear Waste

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Grant 99-35401-7914; $90,000; 2 Years

Many rural communities are vulnerable to opportunity-threat development patterns, in which the potential for collective and individual economic benefits and opportunities of a proposed project are juxtaposed against a variety of possible "threats", including possible negative effects on other existing or potential economic activity, actual or perceived health and safety risks, negative effects on environmental quality, or disruption of social relations within and between communities. Attempts to site a temporary high-level nuclear waste (HLNW) storage facility in the United States illustrate the opportunity-threat conditions that characterize many rural economic development contexts. While the popular view of hazardous facility siting is that communities react negatively to such developments, research points to a number of mitigating factors such as familiarity, local sociocultural context, environmental landscape, economic benefits, and infrastructural improvements that can significantly influence community response. Following failed attempts by other Indian groups, the Skull Valley Goshutes in Utah have proposed to host a temporary HLNW storage facility. In addition to stimulating local concerns about risk, compensation, equity, cultural change, and sovereignty, the Goshutes' proposed facility would be centered among a complex of operating hazardous facilities within a rural area that is highly experienced with economic development activities that reflect the opportunity-threat pattern. Opposition to this proposed facility among members of the non-Indian communities in the surrounding area and among state political leaders suggests a potential for substantial social and political turmoil both within and between local communities ant cultural groups. This study will analyze the rural economic development effects and associated patterns of social change that merge under this opportunity-threat context. Secondary data collection, ethnographic observations, qualitative interviews, and focus groups will be conducted among Indian populations, Euroamerican residents of the area, and political and organizational representatives involved with the project. The primary goal of the research is to determine how opportunity-threat developments affect social relationships and processes within and between communities and community groups.

9901274 Poverty, Labor Markets and the Potential Impact of Welfare Reform on Single Female-Headed Households in the Rural South

Mills, B.F.; Alwang, J.R.; Findeis, J.L.; Jensen, L.
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Grant 99-35401-7706; $105,000, 2 Years 6 Months

The non-metropolitan south exhibits high rates of poverty, particularly among female-headed households with children. A major component of federal assistance to such families is the Federal welfare program. There is hope that welfare reform will benefit such families by providing more incentives to work. Welfare rolls are declining in the south as provisions of welfare reform take hold, but rates of poverty have not shown similar declines. Little solid evidence exists to show how poor households will be affected by welfare reform, particularly their ability to move from welfare to work. The work in this study will provide empirical evidence on the impact of welfare reform on poverty among single female-headed households in the non-metropolitan south. It will examine how people’s decisions whether to work are affected by conditions in the local labor market. It will also show how removal of some of the constraints to working for poor women will change work behavior and the overall well being of their households. The specific objectives are: 1) examine the role of TANF benefits in low-income households; 2) describe the changing face of poverty in the rural south; 3) measure determinants of participation and earnings in southern labor markets; 4) determine how local economic conditions affect low-skill workers; and 5) discuss the policy implications for welfare reform and poverty reduction.

9901249 Older Families in Rural Communities: Personal and Social Influences on Service Use

Roberto, K.A.; Blieszner, R.
Virginia Polytechnic Institute and State University; Center for Gerontology; Blacksburg, VA 24061
Grant 99-35401-7769; $150,000; 2 Years

Given the nations current economic and political climate, it is essential to optimize limited financial and human resources in providing health and community-based services to those living in rural areas. The goal of this project is to learn why older rural adults and their family caregivers use or do not use health and community-based services. We will conduct focus groups with health and community service providers to assist us in the development of questionnaires. Through telephone interviews with 500 elders needing care and approximately 250 family caregivers we will identify not only tangible (e.g., income, availability) but also intangible (e.g., values) variables that influence patterns of help seeking of rural older families. Following the telephone interviews, we will invite approximately 50 elders and 25 caregivers to participate in face-to-face in-depth interviews to verify and expand upon the information gathered during the telephone interviews. Upon completion of data analysis, we will reconvene the focus groups with the health and community service providers to respond to the service related issues raised by the elders and their family providers to gain a better understanding of the role of services in maintaining and enhancing the quality of life of older families living in rural communities. From the interviews and focus groups, we will develop and disseminate Policy Briefs for policy-makers and health and community-based service providers that will inform them as to the mix of both direct support to elders and resources needed to cultivate and enhance the informal care system available to rural elders.

9901272 The Impacts of Amenity Values on Agricultural Lands Conservation: Community Preferences and Property Values in the Rocky Mountain Region

McLeod, D; Inman, K.; Coupal, R.
University of Wyoming; Department of Agricultural and Applied Economics; Laramie, WY 82071-3354
Grant 99-35401-7742; $130,000; 2 Years

Rural land and water are important for agricultural production and rural home sites. The Rocky Mountain region is experiencing a historic rate of rural residential development. We will determine the value residents and landowners put on conserving agricultural lands. Data will be collected by a mail survey in a Colorado county. Wildlife, scenery, recreation and location contribute to rural land values. We will determine how factors are leading to conversion of land from agricultural to residential uses in Wyoming and the region. Project results will be made available in partnership with the American Farmland Trust.

ENHANCING VALUE AND USE OF AGRICULTURAL AND FOREST PRODUCTS

The Enhancing Value and Use of Agricultural and Forest Products Division responds to the growing need to enhance the competitive value and quality of U.S. agricultural and forest products. Research in this area builds the scientific base of knowledge to use agricultural and forest materials more fully and effectively. The Division supports both fundamental and applied research on new and improved processes and on development of new uses for agricultural and forest materials. Program Areas in this Division include: Value-Added Products Research encompassing Food and Non-Food Characterization/Process/ Product Research, and Improved Utilization of Wood and Wood Fiber.

FOOD CHARACTERIZATION/PROCESS/PRODUCT RESEARCH

Panel Manager - Dr. Ralph P. Cavalieri, Washington State University
Program Director - Mr. Jeffery L. Conrad

This program area seeks to better understand the properties (physical, chemical and biological) of raw agricultural materials and products related to their quality and processing characteristics and to develop innovative products and processes for better utilization and more efficient conversion of agricultural materials to higher value food products. Specifically, research is supported on value-added food products which contribute to expanded markets for agricultural commodities, lower-cost food products, and a more competitive domestic food industry with expanded export opportunities. This program supports research to increase the quality, utility, convenience, nutrient value, and safety of food products through innovative processing methods. Research providing the basis for development of new food products is also supported.

9901661 Thermal-stable Species Marker Proteins for Detection of Meat Species Adulteration
Hsieh, Y-H.P.; Chen, F-C.
Auburn University; Department of Nutrition and Food Science; Auburn, AL 36849-5605
Grant 99-35503-8135; $140,000; 3 Years

Meat adulterated with undeclared species imposes substantial concern to consumers. Methods for detection of species adulteration are essential for implementation of meat labeling regulations and product quality control. Current polyclonal antibody (Pab) based immunoassays are expensive, unable to achieve quantitative analysis, and require separate antibody reagents for raw and cooked products. The major difficulty has been related to the lack of appropriate species-specific marker proteins for antibody development. Our preliminary results suggest that a 24 kD thermal-stable muscle protein (TSMP) isolated from porcine muscle could be an ideal species marker protein. Monoclonal antibodies (Mabs) raised against this TSMP are monospecific to porcine species, and quantitative detection of pork tissue can be achieved in both raw and cooked meat products. To accelerate the development of much needed Mabs for detection of meat species adulteration, it is important to further characterize this 24 kD TSMP, and continue to search for similar species-specific marker proteins in other meat species. Therefore, the objectives of the proposed research are to: (1) characterize the porcine 24 kD TSMP; (2) identify similar TSMPs specific to beef and lamb; and (3) develop specific Mabs against each species marker protein for immunoassays. The 24 kD porcine TSMP will be purified to characterize the biochemical properties. The similar marker protein will be purified from beef and lamb, and used as immunogens to develop species-specific Mabs. These Mabs can be employed as economic and reliable analytical means in immunoassays and immunosensors for physiological study of muscle proteins and quantitative detection of adulterated meat.

9901575 Infrared Imaging for On-Line Non-Contact Doneness Inspection of Chicken Meat
Tao, Y.; Ibarra, J.; Shultz, J.; Wen, Z.; Johnson, M.
University of Arkansas; Department of Biological and Agricultural Engineering; Fayetteville, AR 72701
Grant 99-35503-8377; $125,000; 2.5 Years

Consumption of ready-to-eat boneless chicken meat by American consumers is increasing annually. Consumers are demanding consistently high-quality, low-cost chicken meat, along with the USDA assurance of food safety. The poultry processing industry faces a constant challenge to achieve the right temperature point in meat pieces on high-volume cooking lines that preserves yield and quality and also complies with USDA regulations.

This project will develop an on-line, non-invasive technology that uses infrared and laser imaging to estimate the internal temperature of meat after cooking and to identify the coldest/hottest spots inside each meat piece on the cooking line. This 100% inspection would replace the current inspection practice which samples random pieces of meat and is slow, invasive, and susceptible to cross-contamination. The results from this study will create a new technology for on-line, non-contact monitoring for poultry meat cooking processes and will aid quality-control personnel to enhance the quality and safety of ready-to-eat boneless chicken products for the American consumer.

9901479 State Transition Effects on the Mechanism of Fissure Formation in Rice Kernels
Siebenmorgen, T.J.; Bautista, R.C.; Wang, Y.-J.
University of Arkansas; Department of Food Science; Fayetteville, AR 72704
Grant 99-35503-8666; $140,000; 3 Years

The overall goal of this project is to increase the quality, value, and profitability of domestic rice production through a fundamental understanding of milling quality losses due to kernel fissures created during the drying process. Our recent research has prompted the hypothesis that fissuring occurrence is directly related to the moisture content gradient in the kernel and the resultant material properties that it exhibits based on the local state of the material. The rice kernel will go through a state transition during drying/tempering from a “glassy” state to a “rubbery” state at a moisture-dependent

temperature known as the glass transition temperature. The physicomechanical properties of the kernel material change dramatically at the glass transition temperature. Although the glass transition temperature of rice can be determined by using a differential scanning calorimeter or a thermomechanical analyzer over a range of moisture contents, the dynamic thermomechanical analyzer provides more accurate and detailed information regarding the viscoclastic and transport properties of rice at different temperatures, moisture contents, and frequencies. We will use the measured diffusion and mechanical property changes resulting from state transition to explain fissure formation.

9901549 Investigating the Basis of Lipid Oxidation Induced Oxymyoglobin Oxidation

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Grant 99-35503-8132; $130,000; 2 Years

The ferrous, oxy-form of myoglobin (OxyMb) provides the desirable cherry-red color critical to consumer acceptance of fresh meat products. Oxidation of OxyMb to ferric brown metmyoglobin (MetMb) is associated with meat discoloration and results in lost value due to price reductions of whole muscle cuts, or grinding for rapid sale. It has been estimated that improved color stability in beef alone could result in $600 million added value to the meat industry annually. Oxidation of lipid in meat leads to rancid flavors and the production of several reactive products. There appears to be a relationship between discoloration and lipid oxidation in meat. We propose to test the hypothesis that a specific group of lipid oxidation products, , -unsaturated aldehydes, bind to OxyMb resulting in altered protein structure and enhanced MetMb formation. The proposed objectives will define the temporal relationship of aldehyde adduction with OxyMb oxidation, establish the specific amino acids that are adducted, and characterize physical alterations in protein structure caused by adduction. The implication is that lipid oxidation products may enhance discoloration in meat by altering the structure of this protein. Results will elucidate the specific effects of adduction on heme protein oxidation and provide a basis for better understanding lipid:protein oxidation interactions critical to functionality.

9901519 High Performance Tangential Flow Filtration for Production of Purified Whey Proteins

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Grant 99-35503-8167; $170,000; 3 Years

Whey is a relatively inexpensive natural source of a wide range of valuable proteins with unique nutritional, functional, and therapeutic properties. However, these unique characteristics are largely unrealized in existing whey products due to adverse interactions between components in these complex mixtures. Several recent studies have demonstrated that High Performance Tangential Flow Filtration can potentially be used for the cost-effective purification of complex protein mixtures. The overall objective of this research is to examine the application of this emerging membrane technology for the purification of individual whey proteins. Protein separations will be achieved in this work by proper control of: (1) transmembrane pressure to achieve the optimal filtration rates, (2) solution pH and ionic strength to maximize differences in the effective hydrodynamic volume of the individual charged proteins, (3) membrane charge to enhance the electrostatic exclusion of the more retained proteins, and (4) diafiltration conditions to achieve the optimal combination of yield and protein purification. Experiments will be performed with model systems consisting of mixtures of individual whey proteins and with actual whey protein concentrates. This research should provide a foundation for the development of new membrane systems for the purification of individual whey proteins with enhanced commercial value. In addition, this work should establish a framework for the application of High Performance Tangential Flow Filtration to the purification of other agriculture protein products (e.g., egg white proteins).

9901679 A Novel Integrated Process for Pilot-Scale Production of Xanthan Gum

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New Investigator Award/Strengthening Award; Grant 99-35503-8664; $140,000; 3 Years

Xanthan gum, the most important microbial polysaccharide, is widely used as a suspending and thickening agent in the food, chemical, and pharmaceutical industries. However, current industrial production using conventional stirred-tank fermentors is energy-intensive and costly. This is mainly because the high viscosity causes agitation and aeration to be difficult and limits the final yield and productivity. The problem encapsulates challenges in both fermentor design and operation, the key areas in fermentation process development. Our previous work has shown that, by using a novel centrifugal, packed-bed bioreactor (CPBR), xanthan productivity can be elevated significantly. Moreover, incorporation of membrane ultrafiltration (UF) to concentrate fermentation broth before alcohol precipitation can save up to 80% of energy used in downstream recovery. In this proposal, we consider not only the engineering challenges inherent in many scale-up operations but also the quality and functionality of the product produced from the new process, mainly the rheological characteristics of xanthan polymers. The overall project goals are to: (1) design and optimize the CPBR for pilot-scale xanthan gum production; (2) engineer the performance of UF process to further improve xanthan gum recovery; and (3)

evaluate and control the quality and functionality of xanthan polymers produced from this process. Not only will this research project improve our knowledge on the scale-up operation of a novel integrated fermentation process for xanthan gum production, but it will also provide insights on critical issues associated with cell metabolism and product formation kinetics.

9901530 Development of Immunoassays for Detection of Almond and Walnut Proteins in Foods

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Grant 99-35503-8513; $180,000; 3 Years

Almonds and walnuts rank number one and two in world tree nut production, and the United States is the leading producer of both. In almonds, a single water-soluble storage protein accounts for 65% of total seed proteins. Albumin, globulin, and glutelin fractions account for 6.8, 17.6, and 70.1% of total walnut protein. According to a recent epidemiological survey, tree nut allergy affects 0.5% of the population, peanut allergy affects 0.6%, and 0.3% reported "nut" allergy but did not distinguish between tree nut or peanut. For some individuals, tree nut allergy is life threatening and fatalities are reported annually. It is important for food processors and regulatory agencies to be able to accurately label foods to ensure the safety of the public and to avoid expensive recalls. The proposed research seeks to develop accurate and reproducible immunoassays that would permit detection and quantification of the major almond and walnut allergens in foods and food products. The long-term goal of the project is to improve food safety. The specific aims of the project are to: (1) identify the major almond and walnut allergens; (2) develop enzyme-linked immunosorbent assays (ELISA) for almond and walnut proteins that maximize sensitivity and specificity; (3) investigate the practical utility of the assay in commercial foods and develop appropriate protocols for almond and walnut protein extraction from various foods for use in these assays; (4) determine the effects of food processing on reactivity of almond and walnut proteins with human allergic antisera.

The results of the proposed research will serve as a model for other tree nut protein research and will improve fundamental understanding of the allergenic proteins in almond and walnuts. Food industry will be able to use ELISA assays that we will develop to detect almonds and walnuts when present in foods and food products.

9901645 Interactions of Modified Pectins with Acidified Milk Proteins

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Grant 99-35503-8668; $137,837; 2 Years

Colloidal stability is a pervasive problem in the food industry. Liquid diet drinks, drinkable yogurts, flavored milks, fruit juice acidified milks, and protein fortified sports drinks are healthful foods that fit into current lifestyles of fast, easily consumed foods. Hydrocolloids, such as pectin, are used as stabilizers, but precipitation, high viscosity and a slick mouthfeel limit consumption. Numerous theories exist as to the mechanism of pectin stabilization of acidified milks, but it is not known which structures of pectin interact with proteins and contribute to stability.

Our hypothesis is that blockwise de-esterification of a discrete block size on pectin will anchor a small portion of pectin by electrostatic interaction and stabilize acidified milk drinks by allowing hydrophobic interaction. We will modify pectin by enzymatic and alkaline saponification and characterize the fractions for total % degree of esterification (DE), distribution of DE (DDE), molecular weight (MW), ratio of uronic acid to rhamnose and neutral sugars. De-esterification of pectin by plant pectinesterase or alkali results in blockwise and random charge distribution, respectively. We will react highly characterized pectic fractions with purified milk proteins (S2+ S1, , -caseins and -lactoglobulin). We chose milk proteins because of the potential application to acidified milk drinks and because the major milk proteins have known and well defined structures.

The nature of pectin should influence the interaction and stability of dispersions. In this research, we will change the ratio of characterized pectins to known proteins and evaluate the size and polydispersity of complexes by light scattering, particle size analysis, and SDS-gel electrophoresis; we will use a probe to quantify hydrophobic interactions. From this research, we should determine which structures of pectin interact with which proteins to form stable dispersions. This will enhance our ability to develop new products and predict performance of pectin as an ingredient.

9901650 Directed Evolution Mutagenesis of Glucoamylase to Increase its Thermostability and Catalytic Rate

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Grant 99-35503-8667; $133,000; 3 Years

We propose to use multiple rounds of random mutagenesis and recombination (directed enzyme evolution) to isolate mutations with increased thermostability in the starch processing enzyme glucoamylase (GA). GA is used to hydrolyze corn starch into glucose in the production of high-glucose corn syrups, and is one of the most heavily used industrial enzymes. High glucose syrup is used industrially both as a fermentation feedstock (i.e., ethanol production) and in the production of high-fructose corn syrup (HFCS), a product that supplies roughly half the United States sweetener market. Two serious

industrial problems with GA are its low catalytic rate and loss of yield due to accumulation of reversion products. GA action at its limit of thermostability (60°C) takes several days to complete hydrolysis, requiring large amounts of tankage in production facilities. The yield of high glucose syrup in starch processing is about 96% due to accumulation of reversion products. At the industrial volumes used in starch processing, the increase of 2% glucose yield to 98% would save millions of dollars of lost product.

We have previously isolated several site-directed mutations that increase GA thermostability. A triple mutant combining three such mutations had increased yield, due to less accumulation of reversion products, and increased specific activity, both apparently the result of reduced flexibility of the active site. Thus, not only can individual thermostable GA mutations be combined, but have the potential to increase both yield and specific activity. We have now developed a plate screen for the direct isolation of random thermostable mutations of GA and propose a full scale random mutagenesis study utilizing multiple cycles of screening and random recombination to push the thermostability phenotype of GA as high as possible. This will improve the enzyme's process characteristics and potentially increase both its yield and catalytic rate.

Increased GA efficiency and yield will lead to cheaper glucose and ultimately to cheaper HFCS. This will strengthen United States agricultural performance since starch for this process comes almost completely from United States corn, while much of the sucrose competing with HFCS is imported. The market for glucose and HFCS is immense; in 1997, 11.6 million tons (dry) were produced from approximately 790 million bushels of corn, utilizing 8.4% of the total United States corn crop.

9901672 Maximizing Health Benefits of Crucifers

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Grant 99-35503-7010; $185,000; 3 Years

For over a decade, Americans have frequently omitted items such as salt and cholesterol from their diet in an attempt to improve their health. Today, the public aims to reduce the risk for chronic diseases by including specific items in their diet. While some information is purely anecdotal, laboratory and epidemiological studies support the benefit of certain dietary inclusions. For example, a diet rich in vegetables, most particularly cruciferous vegetables such as broccoli and cabbage, decreases one's risk for cancer. Yet not all cruciferous vegetables are equally effective. We found that the precursor for a major health-promoting component in broccoli varied in content by 100-fold across 50 varieties of broccoli surveyed. Furthermore, we found that depending upon food preparation conditions, this precursor may break down to an inactive component, rather than forming the health-promoting component, sulforaphane. We propose two studies. The first will characterize preparation techniques needed to maximize formation of sulforaphane from its precursor. The second study will develop simple methods for estimating relative health benefit that can be used for quality control. The information generated will enable people to make wise choices to improve their health, providing information about which foods and food products are most effective. Past decisions about which varieties to grow have been based mainly upon yield and growth habit. If information were available on the superior health benefit of certain varieties, the food and agricultural industries would find added value in offering these varieties.

9901546 Control of Starch Modification

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Grant 99-35503-8665; $125,000; 2 Years

Most corn starch used commercially in either food or industrial products has been chemically modified to improve its usefulness. In prepared food products, various modified food starches are used. Chemical modifications are also employed in preparing starch products for making paper and for sizing textile fibers, the main non-food applications of starch. Starch occurs in the form of granules, which are relatively dense, partially crystalline, insoluble packages of starch molecules. Chemical modification of starch is done on such native granular starch. After many studies over decades, the starch granule remains beautifully mysterious. If new or improved products are to be made, control of reactions within granules will likely be required because it is almost certain that no new chemical reagents will be approved for starch modification, either for use in foods or in papermaking, and allowable levels of currently approved reagents will not be increased. So control of reaction patterns within granules is an option that must be explored. Being able to determine the pattern of reagent penetration into granules and where reactions occur in granules and on starch molecules is the first step in being able to control modification processes and, thereby, to produce higher value modified starches without changing the type or amount of reagent used. This is the objective of this research.

9901658 Genetic and Environment Effects on Wheat Protein Quality During Grain Development

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Grant 99-35503-8670; $140,000; 2 Years

Grain protein composition of four wheat varieties each having two genetically similar lines but differing at only one gene locus will be measured at three day intervals after anthesis (flowering) until maturity. The pairs have been chosen on the basis of contrasting quality attributes associated with known differences in glutenin subunits coded by the different genes. These glutenin subunits join through disulfide bonds during grain development to form large glutenin molecules that determine dough strength and breadmaking potential. The basic promise of the work is that differences in quality of the mature grain are determined by events that occur during grain development. Three main aims will be pursued. First, an explanation for the contrasting effects of the glutenin subunits on quality will be sought in terms of their temporal accumulation and polymerization, thus providing a greater understanding of the genetic basis for quality differences. This will have application in breeding of new improved varieties. Second, the same measurements will be carried out on two of the varieties using different temperature regimes. The aim will be to investigate the reasons for the loss of dough strength caused by high temperatures in the wheat field prior to harvesting. If the causes could be better understood, this should assist in developing varieties that are resistant to heat stress, thus providing processors with greater consistency of quality. Third, the structure of the precursors of the large glutenin molecules will be studied to give information on how glutenin is assembled from its subunits and thus a clearer picture of glutenin structure.

9901529 Modification of Physical and Functional Properties of Fish Proteins by Low pH

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Grant 99-35503-8285; $160,000; 3 Years

A process has been developed to produce food proteins for humans from low value fish muscle sources. The process solubilizes the proteins in acid and allows both insoluble and soluble impurities to be removed. The isolated food proteins make excellent gels. To fully realize the potential of this process in converting low value fish proteins directly into high value food proteins, the effect of the acid conditions on the properties of the food proteins will be determined to maximize the utility of the protein isolates. Also, the effect of the acid conditions on the tendency of the fish lipids to oxidize and bring about off-flavors and odors will be assessed. Techniques will be evaluated to overcome problems that are observed. This research has the potential to allow the 50 million metric tons of fish currently used to produce animal feeds to be used directly as human foods, as well as providing a means of adding high value to fish processing by-products such as skeletal racks and trimmings.

9901521 Impact of Emulsifiers on the Oxidative Stability of Lipid Dispersions

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Grant 99-35503-8232; $140,000; 3 Years

Current dietary guidelines recommend increased consumption of polyunsaturated fats and omega-3 fatty acids. Delivery of these fats to the general public could be accomplished by their incorporation into food emulsions such as dairy products, mayonnaise, margarine, soups, sauces, baby foods, and beverages. Food emulsions are formed by the dispersion of lipids into water or visa versa. Unfortunately, food emulsions high in unsaturated fats can quickly go rancid due to metals in the water phase interacting with the lipids resulting in an unpalatable product. Therefore, the overall objective of this research will be to develop technologies to engineer the interface of the emulsified lipids so that interactions between metals and lipids are minimized. This will be accomplished by studying how emulsifiers impact lipid peroxides-metal interactions, partitioning of lipid-soluble antioxidants into the water phase and regeneration of lipid-soluble antioxidants by water-soluble reducing compounds. Studies will be conducted using synthetic emulsifiers, phospholipids and proteins as emulsifiers. If technologies can be developed to engineer emulsified lipid droplets to decrease the development of rancidity, this would enable food manufacturers to create foods in which saturated fats were replaced by polyunsaturated fats resulting in the production of healthier food products.

9901676 Antioxidant, Anti-inflammatory and Anticancer Compounds from Tart Cherries

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Grant 99-35503-8147; $210,000; 3 Years

Tart cherries as a commodity have suffered in recent years from over production and consequent low economic returns for producers. Our preliminary experiments have shown that whole cherries and cherry compounds are inexpensive and valuable food ingredients in processed meat products. The thrust of this project is to identify and study compounds from tart cherries which will be used to improve quality and safety of existing value-added foods which potentially will benefit

human health. This will improve cherry utilization in the USA and provide tart cherry growers with increased returns for their product. The specific objectives of our proposal are as follows: (1) isolate and identify tart cherry chemical components and study their potential, in model systems, to act as antioxidants, to prevent inflammation, and to protect against cancer and (2) to determine if tart cherry anthocyanins, phenolic compounds rich in tart cherries, can reduce the incidence and burden of intestinal tumors in Min mice, an animal model of human colon cancer. The proposed research is expected to demonstrate long-term health effects derived from the consumption of tart cherry products. Overall, this line of research is expected to lead to a greater consumption of cherry-containing foods by humans and increase the demand for cherries and value-added cherry products to be used in human foods.

9901654 Sixth Symposium on Lactic Acid Bacteria: Genetics, Metabolism, and Applications

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Grant 99-35503-8118; $8,000; 1 Year

Funds are requested in support of the Sixth Symposium on Lactic Acid Bacteria -Genetics Metabolism and Applications to be held in Veldhoven, the Netherlands, on September 19-23, 1999. This triannual international meeting is held on the lactic acid bacteria (LAB), microorganisms which are intimately involved in food fermentation and constitute a major portion of the human diet since ancient times. LAB play an essential role in the preservation of raw food and contribute to the nutritional, organoleptic and health-promoting properties of foods for humans and animals. Beyond their traditional roles, the LAB are now being exploited for a variety of medical, pharmaceutical, and biotechnological applications; for example expression of vaccines, antimicrobials, industrial enzymes, and bioactive compounds. These old and new critical roles for LAB have lead to an explosion of scientific interest and industrial activity over the past 20 years. The full invited lectures and extended abstracts of the other lectures will be published in a special issue of Antonie van Leeuwenhoek. The number of participants in the conference has increased from 175 in 1983 to 540 in 1996, with US representation near 6% of the participants over the 16 year history. U.S. representation in 1999 is among # the highest in the history of the conference, with five of seventeen plenary lectures to be given by U.S. speakers. This proposal requests $8,000 in funds from the NRIGCP value-added program to aid in the support of expenses for US speakers who will present plenary lectures at the 6th LAB symposium.

9901696 Adding Value to Lower Quality Beef Muscles

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Grant 99-35503-8437; $130,000; 2 Years

Since 1985, beef's share of total meat consumption in the United States has declined from 49% to 39%. However, total meat consumption has increased from 233 to 249 lb, due primarily to increased poultry consumption (from 25 to 33% of total meat). One of the factors implicated as contributing to beef's decline in market share is inconsistency in eating quality resulting in consumer dissatisfaction with the value they received per dollar expended. The high quality cuts of beef represent less than 10% of the carcass, thus, a majority of cuts come from lower quality muscles. Because many consumers cannot afford to buy high quality meats, they are frequently dissatisfied with beef. A recent survey concluded that almost 40% of the round and chuck cuts were unacceptable in tenderness. Many consumers would be willing to pay beef's comparatively higher price if beef could consistently deliver a high quality product. Our objective is to determine the relative contribution of the three primary sources of variation in the tenderness of different muscles (connective tissue, muscle shortening, and protein degradation during aging). This information would enable the beef industries to develop cut specific tenderization strategies, thereby improving tenderness, value, and consumer satisfaction for many cuts currently marketed at reduced prices because of poor eating quality. This information also would facilitate the development of further processed, convenient, pre-cooked or short preparation products that are critical for beef to compete effectively for market share given the current trends in consumer needs and wants.

9901522 Metabolism of Strawberry Fruit with Different Postharvest Tolerances to High CO2

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Grant 99-35503-8165; $140,000; 3 Years

Storage potential and therefore increased market penetration, both nationally and internationally, is limited for many horticultural commodities. Understanding of the metabolic processes involved in tolerance to CO_2 in the storage atmosphere must be improved. Lack of knowledge about the factors affecting the balance between beneficial and detrimental responses to the gas will limit future uses of CO_2-based technologies to extend storage periods of horticultural products. Strawberry fruit provide an excellent system to study these processes because while they can be tolerant to CO_2, we have identified two groups of cultivars with markedly different metabolic responses to high (20%) concentrations of the gas. One group accumulates the fermentation products, acetaldehyde and ethanol, within a few days of exposure to the gas, whereas

the other does not. The group that does not accumulate fermentation products, however, accumulates much greater levels of succinate. Accumulation of this TCA cycle intermediate is a common response to CO_2 treatment, usually ascribed to inhibition of succinate dehydrogenase activity by the gas, but identification of a differential effect related to accumulation of fermentation products is novel. In this study we will increase our understanding of mechanisms by which CO_2 influence fruit metabolism, by assessing how carbon flux occurs through the glycolytic, TCA cycle, fermentation, and other pathways in cultivars representing each group. The long-term aim of research initiated here is to increase understanding of how responses of fruit to elevated CO_2 could be modified and thus lead to increased storage potential.

9901523 Process Dynamics and Mechanics of Expanded Cellular and Microcellular Extrudates

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Grant 99-35503-8264; $160,000; 3 Years

Extrusion cooking is a widely used, continuous process of choice for manufacturing low density, expanded food products of different porosity like breakfast cereals and snack foods. The market for and value of such products will be significantly enhanced if the process could be made amenable to utilization of nutritious but heat and shear sensitive ingredients and the process variables could be quantitatively predicted to produce products with desirable porous cellular and microcellular structures and texture. While of crucial relevance, the ability to make extrudates of defined textural attributes such as crunchiness and porosity using conventional extrusion processes has remained a challenge.

The goal of this research is to produce and characterize starch-based composite microcellular extrudates of variable porosity, cell density and mechanical properties using supercritical (high-pressure) carbon dioxide instead of steam as a blowing agent. Current research in our laboratory has successfully established the efficacy of such a process. The proposed research would involve applications of nucleation and bubble-dynamics theories to predict and quantify the effects die pressure, pressure drop rates and temperature on the phsico-chemical, structural and mechanical properties of extrudates. Studies will be undertaken to develop correlations for a better understanding of structure-function relationship in porous foods made by cooking and supercritical fluid extrusion. The fundamental knowledge obtained will also be applicable to non-food cellular products.

9901680 A Polymer Science Approach to Evaluating the Strength of Wheat Flour Doughs and Glutens

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Grant 99-35503-8577; $150,704; 3 Years

A common requirement of either Durum or bread wheat is the ability to form a dough with the proper flow properties. This allows for shaping by extrusion or sheeting of pasta and noodle products, or the development of desirable baked goods due to expansion of the dough during baking, respectively. However, a better understanding of why different wheat flours produce doughs with widely varying mixing and flow properties is needed to guide crop improvements, new process development, and better overall utilization of wheat flour. The rationale for this proposal is that the unique functional properties of wheat flour doughs are due primarily to the hydrated protein fraction of the dough (gluten), and specifically to the polymeric nature of gluten. Thus, many of the differences in quality of different wheat cultivars and the processing properties of their doughs are likely due to differences in the polymeric properties of their respective glutens. Therefore, the focus of this research proposal is on the characterization of the polymeric properties of wheat flour doughs and glutens using the same fundamental techniques commonly used for synthetic polymers. These techniques have not generally been applied to food biopolymer systems. In particular, the stress relaxation spectrum will be used as the basic characterization of the flow properties. It is expected that information contained in the spectrum will provide a linkage between the molecular structures in a dough or gluten and their practical use in baking or pasta-making. Ultimately, this new knowledge should increase the value of wheat flour.

9901642 Resistant Starches from High-Amylose Maize Starches

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Grant 99-35503-8133; $170,000; 3 Years

Until recently starch was assumed to be completely digestible. It is now well known that for cereal starches, susceptibility to amylase action is variably less-than-complete as a result of thermal treatments during food processing and manufacturing. Starch that is not digested is termed “resistant starch” (RS) and behaves in some respects like dietary fiber. Thus it may be desirable to enhance the level of RS in the diet.

RS that is generated by thermal treatment is termed type 3 RS, and it is stable to most further thermal processing. Some starches (from potato, banana, and high-amylose maize) are partially enzyme-resistant in their unprocessed, native granular form. This form of RS is termed type 2 RS. When potato or banana starch is heated to 100°C, the original enzyme resistance is lost. Unlike potato or banana starch, starch from high-amylose maize is incompletely cooked at 100°C and

retains much of its granular form and enzyme resistance. This behavior makes untreated high-amylose maize starch a unique ingredient for use in manufactured foods. When high-amylose maize starch is processed at 140°C or higher, it loses granular form and much of its original enzyme resistance. Nevertheless, high-amylose maize starch is the preferred starting mateial for commercial production of type 3 RS. This work will explore the nature of the unique RS from unprocessed high-amylose maize, and investigate how thermal treatments might be applied (1) to enhance the level of type 2 RS, and (2) to optimize production of type 3 RS.

9901570 Rational Design of Monoacylated Anthocyanins for Increased Color Stability

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Grant 99-35503-8231; $240,000; 3 Years

There is a well-recognized need to find replacements for a number of synthetic dyestuffs in the food/pharmaceutical/nutraceutical industries, as certain compounds currently in use have raised concerns about safety. Our research is directed toward developing certain acylated anthocyanins that have increased color stability so that they might be used as colorants for foodstuffs. Anthocyanins are natural, plant-derived nongreen colorants.

In the previous project period we developed the biotechnology to produce a number of monoacylated anthocyanins and demonstrated that these compounds had greater color stability (some 8-10-fold) at pH 6 than their nonacylated counterparts. These compounds were prepared semibiosynthetically: aromatic carboxylic acids were added to the cell--suspension culture of the wild carrot, and, for a large number of acids, the 6-O-acylated anthocyanins were isolated and purified. Studies of the K_h of hydration (which results in color loss) allowed us to probe the stereoelectronic factors which stabilize anthocyanins to color loss. Molecular modeling studies, coupled with NMR spectroscopy, allowed us to for the first time to gain insight into the molecular dynamics of these compounds and understand at the molecular level the mechanisms for color stability.

These developments, taken together, now allow us to launch a program, based on sound scientific principles, to design and carry out the biosynthesis of anthocyanins that will have enhanced color stability. Our methods will be to (a) design certain acylated anthocyanins and model these by computer; (b) biosynthesize those holding most promise for color stabilization; (c) evaluate these for color stability in aqueous media at pH 6; (d) optimize the production of the most promising candidate(s) for possible commercial production.

9901556 Production of Gamma-Linolenic and Octadecatetracnoic Acid in Crop Plants

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Grant 99-35503-8669; $115,000; 2 Years

Gamma-linolenic acid (GLA) and octadecatetraenoic acid (OTA) are polyunsaturated fatty acids that play important roles in both human and animal nutrition. They are used by mammals in the synthesis of several classes of regulatory molecules. The synthesis of GLA or OTA by organisms requires an enzyme called $\triangle^6$-desaturase. It converts specific precursor fatty acids into GLA or OTA. While humans and other mammals are able to synthesize GLA and OTA, $\triangle^6$-desaturase activity is, in certain situations, too low to maintain desired levels of these fatty acids. Often, dietary supplimentation helps correct this problem. For example, dietary GLA has been demonstrated to benefit atopic eczema in infants, rheumatoid arthritis, high blood pressure, hyperlipidemia, and diabetic neuropathy. OTA is of potential benefit in certain cardiovascular diseases and nephrocalcinosis.

Unfortunately, while several US crops produce abundant yields of fatty acid rich oil, these plants lack the $\triangle^6$-desaturase enzyme required for GLA or OTA production. Adding the ability to produce GLA or OTA would increase the value of these crops by virtue of improved medical and nutritional utility. We have isolated a plant gene encoding $\triangle^6$-desaturase. We will express this gene in maize-seeds which are rich in the precursor to GLA. This should result in corn seed oil containing significant amounts of GLA. We will also express our gene in maize leaves which produce the precursor to OTA. These leaves used as animal forage could produce milk and meat with increased OTA content.

9901675 Measurement and Control of Fruit and Vegetable Impact Sensitivity

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Grant 99-35503-8256; $130,000; 2 Years

The long-term goals of this research are (1) to reduce impact-related damage in the fruit and vegetable industry by learning how to handle and condition these commodities so that they are less susceptible to damage by impacts occurring during harvesting, and transport; and (2) To develop practical assessment and monitoring tools that growers and processors can use to assess and then adjust commodity conditioning and so maintain high food quality.

Supporting Objectives are to (1) measure influences of temperature, hydration level, strain rate, and maturity on impact properties of pears and apples, and then compare these with potato tubers to; (2) develop a general model using these properties to predict the impact bruising of a range of fruits and vegetables and to control that bruising by managing the growing and conditioning of each commodity; (3) design and test a portable device for assessing commodity condition; and develop a basis for non-destructive bruise detection.

This research will lead to better cultural, conditioning, and handling practices, as well as better variety selection for fruits and vegetables. The result will be to reduce bruising and waste, improve efficiency, and improve quality and economy to the consumer.

9901670 Biopolymer-Biopolymer Interactions at Interfaces and its Impact on Food Colloid Stability

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Grant 99-35503-8527; $140,000; 3 Years

Plant and animal proteins are increasingly being used as macromolecular surfactants in novel dispersion-type fabricated food products. Because a typical food product contains several types of polymeric materials, such as proteins and polysaccharides, the stability of food colloids is dependent on the nature of interaction among the constituent polymeric materials. For instance, biopolymer-biopolymer interactions generally are thermodynamically incompatible. This may result in two-dimensional phase separation in mixed biopolymer films and cause instability in food foams and emulsions. A fundamental understanding of the nature and intensity of interactions between various proteins and protein-polysaccharide mixtures at the air-water and oil-water interfaces is critically needed. This would lead to rational designing of ingredient formulations of protein/protein and protein/polysaccharide mixtures that would provide improved stability to food colloids in fabricated foods. This would also enable increased utilization of plant proteins in human foods. In the proposed project, a theoretical framework for quantitative analysis of biopolymer-biopolymer interactions at air-water and oil-water interfaces will be developed. Using this framework, incompatibility between several proteins and protein-polysaccharide systems at the air-water and oil-water interfaces will be studied to develop a comprehensive understanding of this phenomenon. In addition, an epifluorescence microscopic method will be used to analyze conditions that cause two-dimensional phase separation in mixed biopolymer films at interfaces. The knowledge gained from these extensive studies can be readily used in commercial ingredient formulations to create ideal protein and protein/polysaccharide mixtures that would produce stable food foams and emulsions.

9901536 Control of Reaction Selectivity of Lipases in Microaqueous Media

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Grant 99-35503-8166; $160,000; 3 Years

Despite a domestic consumption rate of 7 million metric tons, the fats and oils processing sector contributes the least (2.3%) of 9 commodity sectors to the total value-addition output of the food processing industry. The use of lipases for transforming lipids into highly functional and value-added specialty products for foods and related applications offers enormous potential (estimated global market in 10's of $billions). Understanding and controlling the reaction selectivity of lipases will allow for cogent bioprocessing strategies to be developed for transforming lipids into these specialty products. This project seeks to develop a comprehensive account of selectivity patterns of selected lipases (each possessing unique selectivity features) for the various types of reactions that could lead to the assembly of value-added "structured" edible lipids (primarily triacylglycerols, TAGs). Lipase selectivity for naturally occurring and abundant fatty acids will be determined for each reaction step leading to the assembly of structured TAGs. In addition, lipase selectivity patterns will be assessed for alternative reaction schemes that could be potentially used to prepare structured TAGs in commercial practice. A critical facet of these analyses is the elucidation of how lipase selectivity patterns change during the course of each of the three consecutive steps required to prepare a structured TAG from the basic building blocks of fatty acids and glycerol. Finally, established patterns of lipase selectivity will be used to design and execute lipid transformations, intended to prepare specific examples of structured TAG with unique functionality, as representative of value-added processes of potential commercial interest.

9901576 Structure-Functionality Relationships in Lipid Systems

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Grant 99-35503-8134; $150,000; 3 Years

The quality, texture and shelf stability of lipid-based food products, such as butter, margarine, shortenings, chocolate (and others), are dependent to some extent on the crystalline microstructure of the lipid phase. The number, size distribution, polymorphic form, shape and network structure of the lipid crystals (which together represent crystalline microstructure) are controlled during processing through a combination of formulation and process parameters. It is this crystalline microstructure that determines the mechanical properties (hardness, snap, spreadability, etc.) and, in part, shelf stability of the final product. In this project, we will carefully control the composition and processing conditions of model lipid systems to produce products with different crystalline microstructures. Different classes of lipids (saturated, monounsaturated, etc.) will be added at different ratios to produce a variety of lipid model systems. These lipids will be crystallized in a two step process that simulates a commercial process to produce a semi-solid material (like margarine or butter). The crystalline structure of the semi-solid product material will be quantified by using several microscopy techniques (confocal, polarized light, electron). The semi-solid materials will then be analyzed for their mechanical properties (storage and loss moduli) as well as empirical measures like hardness, snap, and spreadability. Our ultimate goal is to develop quantitative relationships between crystalline microstructure and the mechanical properties in these model lipid systems. In this way, we will ultimately improve the control of processing conditions for natural fats and oils to generate the desired physical characteristics in lipid-based food products.

NON-FOOD CHARACTERIZATION/PROCESS/PRODUCT RESEARCH

Panel Manager - Dr. Gregory F. Payne, University of Maryland
Program Director - Mr. Jeffery L. Conrad

Agricultural commodities can provide the raw materials for production of numerous industrial and consumer products such as lubricants, fuels, paints, detergents, biodegradable polymers, textile fibers, fiber composites, pharmaceuticals, and various other commodity and specialty chemicals. The Non-Food Characterization/ Process/Product Research program supports research on improved methods for producing existing non-food, agriculturally-derived products and on developing new, non-food uses for agricultural commodities. Research seeks to better understand properties of agricultural materials related to their quality, value, and processing characteristics and to develop innovative products and processes for conversion of agricultural materials to non-food products.

This program also supports biofuels research directed toward understanding and overcoming factors which limit the technical and economic efficiency of production of alcohol fuels and biodiesel. Supported research focuses on the physiological, microbiological, biochemical, and genetic processes and mechanisms controlling the biological conversion of agriculturally important biomass material to alcohol fuels.

9901714 Extracellular Targeting and Improved Recovery of Proteins from Transgenic Tobacco

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Grant 99-35504-7798; $160,000; 2 Years

Plant-based systems for production of human therapeutic proteins offer a number of advantages over current production technologies based on eucaryotic systems such as mammalian cultures or transgenic animals. These advantages include safety, lower capital requirements, lower production costs and ease of scale-up.

The objectives of this multidisciplinary research program are to utilize molecular biology approaches to target recombinant proteins to the extracellular space and to develop novel bioprocessing techniques to recover the protein without requiring complete homogenization of the plant. As a model system we will study the expression of trichosanthin, a plant ribosome-inactivating protein (RIP) found in the root tubers of the Chinese medicinal plant *Trichosanthes kirilowii*, in transgenic tobacco. It is a high value compound with potential applications as an antiviral therapeutic for the treatment of AIDS, immunotoxins for treatment of stomach and lung cancers, biocontrol agent for the delay or prevention of plant viral infection, and improved pathogen resistance in transgenic crops. In the native plant, trichosanthin is formed as a preproprotein with a N-terminal signal sequence and C-terminal extension. We will investigate the roles of these sequences on the subcellular localization and bioactivity of the recombinant protein in transgenic tobacco in an effort to identify generic targeting sequences which would result in high level expression of biologically active protein in the intercellular spaces. Recovery of the recombinant protein from tobacco leaf tissue without extensive cell disruption would not only reduce recovery costs and simplify purification but it would also minimize degradation and denaturation of the product during recovery. We plan to investigate the potential for using vacuum infiltration bioprocessing techniques for recovery of extracellular proteins from transgenic tobacco leaf tissue.

9901434 Development of Corn-Based Edible Vaccines for Livestock

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Grant 99-35504-7799; $160,000; 3 Years

One of the most promising technologies made possible by plant transformation is the development of feed-based oral vaccines for livestock. Genes encoding oral vaccines can be expressed in key feed crops such as corn, making it possible to immunize livestock against disease by simply including this transgenic corn in their normal rations. In this way, the expense and labor involved in vaccinating livestock will be greatly reduced. We propose to lay the foundation for production of these vaccines by developing an adjuvant-based vaccine delivery system in transgenic corn. *E. coli* heat labile enterotoxin subunit B (LT-B) is one such adjuvant which is known to stimulate mucosal immunity and has been used to develop effective oral vaccines.

Our first objective is to evaluate a synthetic LT-B gene for expression in transgenic corn tissue. Under our second objective, we will direct the gene into corn seed by placing it under a seed specific regulatory element and appropriate cellular targeting elements. We will produce transgenic corn with a series of gene/regulatory element combinations and compare the LT-B gene expression levels in corn kernel. Our third objective is to fuse an antigen to the LT-B and use it as a vaccine delivery system. Our first antigen will be from porcine Transmissible Gastroenteritis Virus (TGEV), the causative agent of a major swine disease. Transgenic plants that express antigens in their edible tissue might be used as an inexpensive oral-vaccine production and delivery system.

9901452 The International Workshop on the Regulation of Metabolism, Genetics and Development of the Solvent- and Acid-forming Clostridia

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Grant 99-35504-7916; $5,000; 1 Year

The objectives of this bi-annual workshop are to bring together researchers from around the world that are currently working with the biotechnologically important solvent and acid producing clostridia. This meeting is regarded as a truly international meeting whose venue allows for the active participation of a multi-disciplinary group of researchers, from microbiologists and biochemists to engineers interested in molecular genetics and industrial scale-up of the solventogenic clostridia for industrial fermentation. The meeting format will provide an informal setting for comprehensive 30 minute oral research presentations as well as poster sessions. The focus for this upcoming meeting will be to encourage attendance/participation by young scientists, graduate students and post-doctoral fellows. The *Clostridium* VI workshop will take place Friday and Saturday, May 26 and 27, 2000 on the campus of the University of Illinois in Champaign Urbana. This meeting is scheduled to coincide with the ASM General Meeting which will be held in Los Angeles on May 21-25, 2000.

9901458 Production of Cellulases by Repeated Extraction and Fermentation

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Grant 99-35504-7826; $120,000; 2 Years

This project is to investigate solid phase fermentation for producing cellulases at a low cost. With an estimated low enzyme cost, ethanol manufactured from lignocellulosics will cost less than $0.80 per gallon. Other higher-priced, value added chemicals and materials from renewable resources can become even more competitive in the market place, creating new industries for American agriculture.

Solid phase fermentation has two technical difficulties in slow heat dissipation and low air supply. Heat generated by the microbial cells will raise bed temperature, which can kill the microbes prematurely. In this project, an idea called pressure pulsation will be tested to overcome the technical difficulties. The fermentation will be carried out in an enclosed chamber. The gas pressure in the chamber will first go up and then down, creating a pressure pulsation cycle. When the chamber pressure is up, air is forced; when the pressure is done, gas will come out of the voids. This pressure pulsation creates convective gas flows, which enhance air supply and heat dissipation and thus microbial growth and enzyme production. Once the microbes are fully grown, sterilized water is pumped into the chamber to extract enzymes. After removal of the liquid extract, fermentation is re-started by re-introducing air flow. Fermentation and extraction are repeated in 24 hour intervals. In preliminary experiments, this repeated fermentation and extraction resulted in an impressively high cellulases productivity of 806 filter paper units per hour per liter of solid bed, reducing the enzyme cost drastically.

9901451 Structure-Property Relationships in Polyurethanes Based on Vegetable Oils

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Strengthening Award; Grant 99-35504-7873; $165,000; 3 Years

Agricultural products are a source of many valuable chemicals. Vegetable oils have a number of excellent properties, which could be utilized in producing valuable polymeric materials. They can be transformed into polyols for general polyurethane use. Polyurethanes are a group of polymeric materials with many applications. Their current consumption exceeds 2.4 million tons per year in the United States. Replacing petrochemical raw materials with renewable resources such as vegetable oils is of significant economic, environmental and strategic importance, particularly in United States -- the world's largest producer of vegetable oils. One of the basic problems in using raw materials of vegetable origin, such as oils, is that their structure varies more than that of synthetic chemicals. This is reflected in the variability of structure and properties the polymer products based on vegetable oils.

The objective of the project is to study the effect of polyurethane network structure on properties of two groups of materials: rigid foams and cast resins. The network structure will be varied by varying the type of oils, the type and content of hydroxyl groups in the polyols, and the type of isocyanates. The oils to be used will include commercial vegetable oils such as soybean, corn, canola, rapeseed, olive, linseed, safflower, sunflower, peanut, cottonseed, and palm oils, as well as model triglycerides. The effect of polyurethane structure on physical, mechanical and electrical properties will be studied. The results of the study will help us control the properties of the oil-based plastic materials in the optimal way.

9901441 High-Value Fuel Additives and Chemicals from Soybean Oil

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Grant 99-35504-8673; $120,000; 2 Years

Soybean oil, castor oil, and other triglyceride feedstocks will be used to synthesize nitrates and evaluate respective performances as diesel fuel additives. The additives, known as cetane improvers, have been reported to reduce NOx, particulate, and hydrocarbon emissions when blended with diesel at concentrations of about 0.1%. In addition, the increased cetane numbers resulting from the addition of cetane improvers to diesel fuel are known to improve cold-start capabilities and result in smoother engine operation.

Previous research has shown that model compounds (relatively pure organic acids such as oleic acid) can be converted to cetane improvers that have >80% of the performance of the petroleum-derived commercial product, 2-ethylhexyl nitrate. Similar products produced from soybean oil and biodiesel (methylester transesterification products of soybean oil) typically perform at <80%of the performance of 2-ethylhexyl nitrate. The research will focus on model compounds and reactions to develop a better understanding of the reactivity of soybean oil and its respective fatty acids. Goals include attaining a better understanding of soybean oil reactivity and to use this knowledge to produce better cetane improvers from soybean oil. The research team is comprised of faculty from the Department of Chemistry and Department of Chemical and Petroleum Engineering at the University of Kansas.

9901468 Bioengineered Emulsifiers

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Grant 99-35504-7915; $200,000; 3 Years

The objective of this program is to bioengineer the structure of a biologically-derived emulsifier, emulsan. Emulsan is a lipopolysaccharide, characterized by a polysaccharide main chain and a variety of fatty acid side chains to provide the hydrophilic/hydrophobic balance. This polymer, produced by a bacterium, is formed in high concentrations when the organism is grown on a variety of hydrocarbons including ethanol. We have used different strategies to manipulate and 'tailor' the structural features of the polymer, resulting in modifications in the fatty acids and thus the nature of the hydrophobic portion of the polymer. The current plans are aimed at optimizing the yield of this bioemulsifier when produced on agricultural feedstocks. We will then address the emulsification properties and structural features of these polymers when produced on these types of energy sources. Additional plans are to further the molecular level understanding of the biosynthesis of the polymer through continuation of the genetic mapping and characterization of the pathway. The direct conversion of agricultural biomass into value-added emulsifiers and surfactants is the main objective of the program. The potential use of these types of compounds in a variety of industries, from food and cosmetic, to oil, environmental and biomedical, is envisioned. In addition, these types of polymers, derived from microorganisms, will be biodegradable. The bioengineering approach thus provides a cradle-to-grave control of polymer production and waste disposal with green chemistry in mind.

9901423 Genetic Engineering of Amylosaccharidases for Agriculture Bioprocessing

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Grant 99-35504-7811; $200,000; 3 Years

A variety of amylosaccharidases are used industrially to produce carbohydrate syrups needed as sweeteners or fermentation feedstocks. Starch bioprocessing requires high temperature stability and activity, properties which are common to thermozymes (i.e., enzymes found in thermophilic and hyperthermophilic microbes). The proposed research deals with understanding the molecular determinants for activity and stability in *Pyrococcus furiosus* a-amylase and amylopullulanase. Their respective genes have been cloned and sequenced, and the recombinant enzymes have been partially characterized in our laboratory. Protein engineering studies utilizing site-directed mutagenesis, DNA shuffling, and enzyme kinetic analyses will be performed to understand structure-function relationships and to design factitious enzymes for use in industrial starch processing systems. We specifically propose to: (1) characterize the active site of *P. furiosus* amylopullulanase in regard to the residues responsible for catalysis and bond cleavage specificity; (2) collaboratively determine the three-dimensional structures of *P. furiosus* amylopullulanase and a-amylase; and (3) identify the residues responsible for high thermoacid stability in *P. furiosus* a-amylase. These studies will expand our understanding of the biochemistry of amylosaccharidase thermozymes and their biotechnological utility in starch processing.

9901711 Processing and Process Modeling of Extrusion Cast Films/Sheets from Agricultural Materials

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Grant 99-35206-7950; $180,000; 3 Years

Packaging films/sheets constitute a sizeable portion of our municipal solid waste and their inherent non-biodegradability is a major source of pollution. In the United States alone, approximately over 1 billion pounds of petroleum-based plastics are used for this purpose. Similar volumes are in use in Europe. Packaging films are made by flat film extrusion using chill roll casting. In this process, the melt from the extruder is used for making large volume, high quality sheets and films continuously. Since it is difficult to cast films from purely agricultural material in the melt state, blends of biodegradable synthetic aliphatic polyester and agricultural polymer such as starch or gluten proteins will be extrusion casted into films. It is expected that approximately 50% of the synthetic polyester could be replaced with natural polymers thus leading to a significant value addition. These blends would serve as an alternative to pure petroleum-based polymers.

The objective of this proposal is to evaluate the physical, morphological, and functional properties of these films as a function of various process conditions and blend compositions. The polyester will be modified by incorporating a functional group capable of reacting with functional groups on natural polymers. Numerical simulation of the calendering process will be attempted using two different classes of rheological constitutive equations. To our knowledge, this is the first attempt to develop process models for calendered sheets. Development and validation of process models would aid in efficient product development and result in a cheaper product.

9901442 Production of Diverse Biodegradable Polymers in a Model Phototroph

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Grant 99-35504-7983; $160,000; 2 Years

For future environmental and economic concerns in the United States it will be beneficial to use solar energy and natural methods to synthesize what are typically chemically-derived, petroleum-based products such as polyester compounds (e.g., plastics). However, to do so will require more basic knowledge in areas related to the synthesis of carbon storage macromolecules like polyhydroxyalkanoates (PHAs) and the metabolic interplay between different carbon polymers like starch (or glycogen) and PHAs. Such advances in our understanding will come from studies on model phototrophs like the ones described here. Photosynthetic bacteria are noted for their metabolic versatility with respect to the use of carbon, energy and nitrogen sources in addition to their biosynthetic capabilities. For example, *Rhodobacter capsulatus* has been shown to naturally produce high levels of glycogen when grown on some sugars while it produces high levels of the plastic, polyhydroxybutyrate (PHB) on other carbon sources like succinate/acetate, fatty acids or acetone. Using genetic screens and selections already developed by the PIs, and the complete genomic sequence of *R.capsulatus*, three objectives are proposed here to understand pathways for glycogen and PHA synthesis in *R.capsulatus*. (1) A strategic genetic analysis will be performed to investigate these pathways and their interrelationships. Mutants in glycogen and PHA synthesis will be constructed and evaluated. (2) The screens and selection techniques will be used to discover genes involved in the PHA biosynthetic pathway(s) and to create altered fluxes. (3) The specific acetone pathway that leads to PHA production will be analyzed.

9901739 Development of *Podophyllum peltatum* as an Alternative Crop for Small Farmers

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Strengthening Award; Grant 99-35504-8261; $140,000; 3 Years

Etoposide and teniposide are semi-synthetic anticancer drugs for which the starting compound is podophyllotoxin, a natural lignan found in *Podophyllum* species. Etoposide has been used for the treatment of lung and testicular cancer, and teniposide is used to treat leukemia. In order for a therapy involving the use of natural products to be successful a reliable drug supply is needed. This is a critical issue for natural products, derived from collections of wild populations. The commercial source of podophyllotoxin is *Podophyllum emodii* Wall. (Syn. P. *hexandrum* Royale), a species native to the Himalayas, which was declared in danger of extinction during the 1989 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). As a result, the export of the raw material of *P. emodii* was prohibited. The resin, however, is still being exported, a practice that contributes to the disappearance of the species. An alternative source could be the North American species *Podophyllum peltatum* L. (mayapple); therefore a survey of the United States' population was done. We have identified and selected some promising accessions. These are high yielding plants, which have been propagated to establish a variety repository. The objectives of our study are:1)to confirm elite genotypes for podophyllotoxin production; 2) to produce healthy propagules and evaluate somaclonal variation; and 3) to determine the effects of pre- and post-harvest treatments on podophyllotoxin yield. We expect our research to have a positive impact on the supply of podophyllotoxin for cancer patients and for mayapple to become an alternative source of income for American farmers.

9901709 Development of an Efficient and Scalable Synthetic Route to D-Glucaric Acid
Kiely, D.
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Strengthening Award; Grant 99-35504-8262; $89,605; 2 Years

The long term goal of this research is to develop new value added products from glucaric acid, a six carbon carbohydrate diacid derived from glucose (dextrose), a major commercial agrochemical from corn starch hydrolysis (corn refining industry). We envision that when a commercial process for producing glucaric becomes available, glucaric acid will become a large scale "stand alone" chemical with a wide range of commercial applications, including those in the food, diet supplement, detergent, cosmetic, and pharmaceutical industries. In addition glucaric acid is the principal renewable resource "building block" for a number of novel synthetic polymers (polyhydroxypolyamides) developed in the PI's lab. These materials are targeted as both biodegradable functional materials (e.g., for use as environmentally friendly components in various manufacturing processes) and building materials (e.g., for use in short term biodegradable plastics applications). The specific goal of this project is to establish an efficient laboratory procedure for the chemical conversion of glucose to glucaric acid that will quickly lead to a pilot plant scale and then industrial scale process. Funding for this project will allow us to evaluate, and hopefully develop a sound lab scale version of the conversion step as part of a projected commercial process for glucaric acid.

9901466 UV-Curable Epoxidized Linseed Oil-Based Ceramer Coating
Soucek, M.D.
North Dakota State University; Department of Polymers and Coatings; Fargo, ND 58105
Grant 99-35504-7852; $160,000; 2 Years

The goal of this study is to control the phase morphology of inorganic/organic hybrid coatings. A secondary goal of this study is to prepare more reactive epoxides using linseed oil as a starting material. Epoxidized linseed oil and titanium based sol-gel precursors will serve as the organic and inorganic phases, respectively. Both the inorganic and organic phases will be cured concomitantly *via* cationic photoinitiator. The curing of inorganic phase, and hence the morphology, will be controlled by relative acid strength, sol-gel reactant, and additives. In addition to acid strength, the curing of the organic phase will be studied as a function of type of epoxide. In all, three types of epoxides will be used as the organic phase: internal glycidyl, cyclohexyl, and norbornyl. It is hoped that by controlling the rate of formation of both the organic and inorganic phases that clear translucent coatings formulations can be UV-cured into white pigmented films.

9901472 Practical Production of Bacterial Cellulose
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Strengthening Award; Grant 99-35504-8011; $70,000; 1 Year

Cohesive cellulose films are formed by *Acetobacter xylinum* in a rotating disk bioreactor with relatively dilute solutions of sugars. The product is pure cellulose as a highly hydrated gel from which the organisms are removed easily. Much of the water can be squeezed out leaving cellulose to dry quickly to a very tough sheet for such applications as making acoustic diaphragms for headphones and loud speakers. The disk reactor has a high surface area for film growth, and a run takes 3 to 4 days in contrast to the old surface culture method that takes 7 to 14 days. Different solid materials can be incorporated into the gel to make gradients of solids, stripes of solids, and bands. There are existing and potential applications for both the wet gels and dry sheets of cellulose and its composites with solids for medicine, electronics, food, and paper. This project will address adapting this system to agricultural practices in the United States and testing more solids that will inspire new applications. Specific goals are obtaining data on which scale up can be based, improving methods for harvesting the product from the disks, defining mixing conditions for good incorporation of solids into the gel as it forms, characterizing properties of gels and dried sheets that are composites of cellulose and various solid particles, surveying new applications, and analyzing costs of the proposed processes.

9901722 Metabolic Engineering of Propionibacterium for Enhanced Propionic Acid Fermentation
Yang, S.T.
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Grant 99-35504-7800; $140,000; 2 Years

Propionic acid is used in the manufacture of herbicides, chemical intermediates, artificial fruit flavors, pharmaceuticals, cellulose acetate propionate, and preservatives for food, animal feed, and grain. The project goal is to develop a process to economically produce propionic acid from low-value agricultural commodities and food processing byproducts, such as corn fiber and corn steep liquor from corn wet-milling and cheese whey from the dairy industry. Propionibacteria usually produce propionate, acetate, succinate, and CO_2 from sugars such as glucose. Conventional fermentation technologies for propionic acid production are limited by low reactor productivity, product yield, and product concentration. Novel metabolic engineering approaches, at both molecular biology and process engineering levels, will be

developed for enhanced propionic acid production by *Propionibacterium acidipropionici*. The proposed work includes: (1) disruption of acetate kinase (ack) and/or phosphotransacetylase (pta) genes to block acetate formation in the dicarboxylic acid pathway; and (2) development of an extractive fermentation process for enhanced propionic acid production. Disruption of ack and pta genes would force cells to use HMP pathway, reduce cell growth, and increase propionate production by an estimated 50% or more. Selectively removing propionate from the fermentation broth by solvent extraction would reduce product inhibition and allow more and faster propionate production. The expected higher productivity, product yield, and final product concentration from an extractive fermentation process using the metabolically engineered bacteria should allow the fermentation produced propionic acid to compete more favorably with that currently made from petrochemical-based synthetic processes.

9901715 Kinetics of Rapid Structural Evolution in Processing of PLA Films Through Fast Spectral Birefringence Technique

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Grant 99-35504-7823; $175,000; 3 Years

The majority of currently available commercial polymers are derived from nonrenewable sources such as petroleum. The world's supply of these non-renewable sources is expected to diminish in the next century, therefore polymers from renewable resources such as those from agricultural products will gradually replace the current ones.

One of the most promising technologies currently under development is the corn based lactic acid which when polymerized yields a tough and strong polymer. This was brought about by the recent technological developments in technologies that resulted in development of industrially acceptable purity lactic acid monomer. The polymers produced, Poly lactic acid and its chemical and physical derivatives (e.g., Copolymers) and physical derivatives (blends with other polymers) are being introduced into the market in the form of variety of packaging materials.

The most advantageous feature of this class of polymers is their ability to biodegrade into environmentally benign by products. This degradation rate can also be controlled through variety of chemical methods-- through copolymerization with other monomers and physical methods-- through blending on the requirements of the applications.

The main aim of this project is to investigate the basic mechanism of deformation of PLA films through the use of newly automated spectral birefringence technique. The ability to measure this parameter that carries information about crystallinity and orientation rapidly allows us to quantify the structural formation mechanism(s) during various stages of product formation thereby helping to optimize the use properties obtained in the final products. In addition this technique is robust enough to be used as on-line structural monitoring tool to be used in a control strategy to set and maintain close tolerances in the structure and consequently the properties of PLA films in commercial manufacturing processes.

9901428 Mixed Culture Transformation of Anaerobic Cellulolytic Bacteria

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New Investigator Award; Grant 99-35504-7830; $100,000; 1.5 Years

Cellulose waste or biomass is a major by-product of agriculture. Cellulosic biomass can potentially be converted to ethanol or other desired products in one step using bacteria able to produce the cellulase enzymes necessary to break down cellulose while simultaneously fermenting the resultant sugars. These bacteria grow without oxygen (anaerobically) at high temperature. Economic studies offer strong support for the potential of such "consolidated bioprocessing," and several lines of evidence support the kinetic and bioenergetic feasibility of this concept. One attractive strategy for developing organisms capable of consolidated bioprocessing is to genetically engineer the end product metabolism of naturally cellulolytic anaerobic bacteria such as *Clostridium thermocellum*. The major impediment to pursuing this strategy is the absence, in spite of considerable effort, of a gene transfer system for *C. thermocellum* or similar cellulolytic anaerobes. Both the literature and preliminary results reported herein support the efficacy of screening many strains from the environment to find one or more actively cellulolytic strains amenable to genetic manipulation. We propose to pursue this by electrotransformation of cellulolytic enrichment cultures followed by isolation and characterization of presumptive transformants, which differs from the conventional approach of isolation followed by transformation. The objectives of the project are to develop a genetic system for one or more actively cellulolytic bacteria as well as to demonstrate and evaluate the mixed culture transformation approach. Successful completion of the project will provide transformable potential type strains for subsequent studies of both applied and fundamental significance.

IMPROVED UTILIZATION OF WOOD AND WOOD FIBER

Panel Manager - Dr Timothy G. Rials, USDA Forest Service, Southern Regional Experiment Station
Program Director - Mr. Jeffery L. Conrad

Improved wood utilization practices depend upon a continually advancing scientific foundation of basic research in wood properties and fundamental components of wood science. This program area supports research that addresses critical barriers to improved wood utilization and that will provide the scientific base from which new research and development can proceed. The major areas of focus include: (1) wood chemistry and biochemistry, (2) physical and mechanical properties of wood and basic wood processing technology, (3) structural wood engineering, and (4) forest engineering research. Innovative approaches to solving fundamental problems in the field of wood science and technology are encouraged.

9902432 Surface Roughness Measurement Using Air-Coupled Ultrasound
Johnson, G.C.; Beall, F.C.
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Grant 99-35504-8063; $98,000; 2 Years

The surface quality of a part determines, to a great extent, its suitability for a particular application. This work will examine the feasibility of using air-coupled ultrasonic waves to measure the surface roughness of wood and wood-based products. Compared to most structural materials, wood is relatively soft and has relatively large surface roughness. These characteristics make the use of traditional techniques for surface texture measurement difficult. Air-coupled ultrasound appears to be a promising technique for surface examination in that the characteristic acoustic features involved are well suited to the geometry being examined. Preliminary work demonstrates that airborne ultrasonic waves can be generated and detected using available equipment, and that rough surfaces have a significant effect on the received waves. State-of-the-art flat and focused transducers and amplifiers will be acquired in order to use higher frequencies than in previous work. Flat transducers will provide an average roughness measure over the surface, while the focused transducers will allow more detailed surface profiles to be obtained. Theoretical work will proceed in parallel with the experiments in identifying appropriate algorithms for obtaining quantitative estimates of surface texture from the measurements. This research will lead to the scientific basis for developing improved methods for measuring surface roughness of wood.

9903969 Reactivity of Pine Trees with Modified Lignins
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Institute of Paper Science and Technology; Atlanta, GA 30318-5794
Grant 99-35103-8365; $160,000; 2 Years

More and more kraft pulp mills are using catalysts, such as anthraquinone (AQ). This increase in use can be attributed to improved mill productivity and a recent reduction in catalyst cost. However, AQ is not the only effective catalyst; dimethylated anthraquinone analogs are more effective than AQ. For example, a soda pulp produced from pine with 0.05% added dimethylated anthraquinone analog had the same kappa number, yield, viscosity, bleachability response, and strength parameters as a soda/0.10% AQ pulp. Similar results were observed for kraft/ and polysulfide/catalyst pulping. Residual dimethyl anthraquinone was present in approximately four times higher amounts than residual AQ in the unbleached pulps. The improved survival may account for the new catayst's greater effectiveness. A simple synthesis of dimethylated anthraquinone analogs is available that could potentially lower the cost of using pulping catalysts.

9902605 Towards Optimal Performance of Cellulose Fiber Networks
Ostoja-Starzewski, M.
Institute of Paper Science and Technology; Atlanta, GA 30318
Strengthening Award; Grant 99-35504-8672; $100,000; 2 Years

The paper industry's competitiveness and environmental concerns require a much improved understanding of paper mechanical properties in terms of microstructure. This insight would allow an optimization of the manufacturing processes and a minimization of the amounts of raw materials going into the paper products. This presents a major challenge in passage from the level of a fiber network to that of an effective continuum. Our strategy of attack is based on a finite element program which is analogous to a quasi-static molecular dynamics, and a three-dimensional fiber network representing paper structure. This program allows a quantitative analysis of a complex spatial system of all the fiber interactions and estimation of relative influences of all the microscale parameters. There are three goals of this proposal: (1) inclusion of fiber plasticity in the model, to enable simulation of elastic-plastic-brittle fracture/damage of fiber networks. (2) studies of effective (scale-dependent) mechanical responses (stiffness and strength/fracture) of fiber networks for a wide range of parameters - e.g., from bad to good formations - and, on that basis, finding of cellulose fiber networks that lead to optimal properties of paper materials, and (3) verification of the model by a comparison of its predictions to deformation patterns observed on sub-

millimeter scales in low-density paper. It is intended to synthesize the results of this program in ready-to-use formulas, maps and software modules that could be used by paper industry, researchers and students.

9902593 Fundamentals of Photobleeding Lignin

Ragauskas, A.J.
Institute of Paper Science and Technology; Atlanta, GA 30318-5794
Grant 99-35103-8603; $90,000; 2 Years

Light induced aging of wood and wood-based materials is an important problem that limits the commercial utilization of wood for many products. Future uses of wood and the development of tree farming could be accelerated if the photoaging of wood materials could be halted or significantly retarded. Recently, our research group has published data demonstrating that acetylated, high-brightness mechanical pulps are photostable when irradiated with office lighting. Preliminary studies indicate the photostabilization effect is due to acetylated lignin that photobleaches wood fibers upon irradiation. Unfortunately, current acetylation procedures preclude the commercial application of this technology. Furthermore, the fundamental processes contributing to the photobleaching effect are unknown. This research program will identify the chemical structures that photostabilize lignin in acetylated high brightness mechanical pulps. The fundamental research goals of this project will be accomplished by fully characterizing, acetylated lignin before and after irradiation. Lignin, isolated from wood fibers will be acetylated and irradiated on cellulose and mechanical pulp test sheets. The photolyzed material will be re-isolated and characterized by UV/Vis, FT-IR, and advanced NMR techniques (lD:'H, ^{13}C, phosphorylation and ^{31}P NMR; 2D: HMQC). In this manner we will be able to identify the chemical structures that contribute to the photostabilization effect. These results will provide a fundamental basis from which practical photostabilization technologies can be developed for wood-based materials.

9902444 Benzenediols and Benzoquinones: Roles in Wood Decay by Brown-Rot Fungi

Crawford, R.; Paszczynski, A.
University of Idaho; Environmental Biotechnology Institute; Moscow, ID 83844-1052
Strengthening Award; Grant 99-35103-7997; $150,000; 2 Years.

Lignocellulose is among the most abundant, renewable natural materials on earth. As wood, lignocellulose provides a versatile building material, an important fuel for many societies, and an important reservoir of carbon for the earth's carbon cycle. The lignin in plant tissue is an important source of stable organic matter in soils, especially undisturbed forest soils, but also most agricultural and grassland soils. Biological decay of wood causes losses of billions of dollars to the United States. Thus, an understanding of the processes involved in biodegradation of lignocellulose is important across many disciplines of science and engineering. One of the major forms of wood decay is fungal brown rot. The specific mechanisms of brown rot are not well understood. The research performed during this project will lead to an improved understanding of biochemical mechanisms of brown rot decay, and should lead to strategies for its control. We will determine whether or not certain benzenediols and benzoquinones produced *de novo* by the brown rot fungus *Gloeophyllum trabeum* are involved in lignocellulose catabolism through promotion of oxidative Fenton’s reactions. We will examine approaches to inhibit these reactions and determine if these approaches can be used to prevent or decrease brown rot decay.

9902358 Investigating the Influence of Voids on the Engineering Constants of Oriented Strandboard

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Grant 99-35103-8298; $98,000; 2 Years

Oriented strandboard (OSB) is a structural wood composite with primary applications as sheathing, overlaid panel siding, I-joist webstock, and single-layer flooring. OSB is made by pressing a loosely packed flake (resin-coated) mat into a panel at a target thickness. While the density of the mat increases during the pressing operation, the final product still contains a substantial volume occupied by voids. The presence of voids in OSB reduces its elastic constants and influences its moisture resistance. In this work, a combined approach of continuum modeling and finite element analysis is proposed to quantify the effect of voids on elastic moduli and moisture expansion coefficients of OSB. Effects of raw material properties, flake orientation distribution, board construction, and voids (size, shape and volume) on the engineering constants will be investigated through model analysis. The experimental phase of the project enables the development of valid flake properties as input to the simulation model and validation of predicted effective engineering constants of porous OSB. Successful completion of the project will advance our understanding of the void structure and its effect on OSB properties, and will also lead to an analytical tool which could be used to optimize the engineering constants for manufacturing structural panels. This will help develop new OSB products (e.g., low density panels) which meet mandatory requirements in stiffness, strength, and stability, while maintaining minimum raw material and manufacturing costs.

9902439 Property Determination of Wood, Wood Strands, and Wood Fibers with IR Analysis

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Grant 99-35103-7972; $l30,000; 2 Years

Modern instrumentation has made it possible to rapidly acquire spectra on large numbers of samples. However, if the speed of these spectroscopic experiments is to be exploited, similarly powerful data analysis tools will be required to uncover subtle relationships between the spectra and material properties. Multivariate chemometric techniques will be used to determine the relationship between properties (mechanical, physical, and chemical) of wood solid, wood flakes, and wood fibers) and their corresponding infrared spectra (FTIR, NIR). The determination of spectral relationships with the mechanical, physical, and chemical properties of solid wood and wood components will allow the development of predictive models which have heretofore been nonexistent. The consequences or this research could be far reaching with regards to the forest products industry: (a) the development of a hand-held portable field unit capable of assessing all major wood properties on standing timber, and (b) the development of on-line equipment which assesses material properties of solid wood and wood components (and thus dynamically altering the manufacturing stream in accordance with these assessed properties). As a result, this research will allow land managers to work in concert with manufacturers of wood based-composites, allow geneticists to make long-range decisions affecting the productivity and quality of future timber stands, and allow forest products manufacturers to produce commodities which are efficient, reliable, and cost-effective.

9902366 A Novel Approach for Determining the Role of Ethylene in Wood Formation

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Grant 99-35103-8071; $165,000; 3 Years

The quality and properties of wood are influenced by the relative abundance of the different cell types that comprise wood. Factors such as the composition of the cell wall, the arrangement and orientation of the cell wall, water content and relative abundance of cell types are just a few of the inherent factors that contribute to the overall structure of wood. Variation in wood structure and physical properties of wood is often influenced by environmental changes. These changes may represent adaptive mechanisms for survival. Circumstantial evidence suggests that the plant phytohormone ethylene plays an important role in wood formation in trees. In this research, the role of ethylene in wood formation in poplars (*Populus*) will be examined using trees that have been genetically engineered to be defective in perceiving ethylene. In theory, aspects of wood formation that rely on ethylene should be altered. Using these genetically engineered poplars, the morphological and anatomical features of wood from one-year-old and two-year-old trees will be examined and compared with the wood from wild-type poplars. In addition, the induction and abundance of reaction wood (tension wood) in mechanically stressed trees of both genetically engineered and wild type poplars will be assayed. As wood and fiber production becomes more dependent upon intensively managed plantations, strategies that provide a genetic means for increasing production, improving quality and enhancing sustainability are needed to enhance the competitiveness of the United States forest products industry. The results of this project will provide the basic knowledge required to develop such strategies.

9902442 Genetic Engineering of Cellulose Biosynthesis in Trees: A Strategy to Cellulose Augmentation and Lignin Reduction

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Grant 99-35103-7986; $215,000; 3 Years

Many of society's fiber, chemical and energy demands are met through the industrial-scale production of cellulose from wood, during which lignin, a cellulose-binding polymer in wood, must be removed at tremendous environmental and process cost. Current wood pulping industry is burdened with low cellulose yield and high process costs to remove lignin. The goal of this project is to develop genetically engineered trees with high cellulose content and low lignin quantity to allow the production of high yield of cellulose from wood with less lignin degrading chemicals and energy. Previously we demonstrated that lignin in trees can be genetically reduced without compromising the structural integrity or growth of trees, and that the deposition of cellulose and lignin may be regulated in a compensatory fashion. Thus, using aspen as a model tree species, we will in this project genetically engineer cellulose biosynthesis to augment the cellulose production with a concomitant reduction of lignin in trees. Aspen cellulose synthesis gene *PtCelA*, the first such gene cloned from a tree species in our lab, will be engineered into aspen trees by using the gene transfer system developed in our lab. Two objectives of this research are: (1) transfer *PtCelA* gene into normal aspen and (2) transfer *PtCelA* gene into our existing aspen engineered with low lignin content. Engineered trees will be maintained in a greenhouse and characterized for their genetic modification, growth, and cellulose and lignin contents during the 3-year project duration. The significance of this research is its potential to result in the production of new tree species that would maximize the output and reduce environmental impact of materials and energy production from wood.

9902423 Foaming of Wood-Fiber/Thermoplastic Composites

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New Investigator/Strengthening Award; Grant 99-35103-8601; $90,000; 2 Years

Wood-fiber/plastic composites make use of cellulosic fibers as fillers and/or reinforcing agents in the plastic matrix. These composites have a lower material cost and a higher stiffness than neat plastics. Although these composites have been commercialized, their potential for use in many industrial applications has been limited because of their brittleness, lower impact resistance, and higher density compared to neat plastics. The potential range of uses for these materials in innovative applications would be expanded if these shortcomings could be overcome. Recently, the concept of creating microcellular foamed structures in the composites as a means to resolve these shortcomings has successfully been demonstrated by the PI. Microcells have been developed in the composites through a batch foaming process by using carbon dioxide as a foaming agent. Because of the generated microvoids, the impact strength, brittleness, and density of the composites were dramatically improved. These enhancements were achieved without significantly compromising other mechanical properties. Despite these promising results, the batch foaming process used to generate cellular foamed structures in the composites cannot now be implemented in the industrial production of foams because it is not cost-effective. A procedure for continuous manufacture of foamed wood-fiber/plastic composites is required for the expanding commercialization of these products and is the focus of this study. The objective of the proposed investigation is to develop the scientific and engineering basis needed to manufacture wood-fiber/plastic composite foams using a continuous extrusion process. Positive results from this study are expected to lead to a new class of materials with unique properties and characteristics. Improving the shortcomings of wood-fiber/plastic composites will extend their utilization in a large number of innovative applications and increase the value-added utilization of wood.

9902751 Friction-Damped Energy-Dissipating Timber Connectors: Pilot Tests and Studies

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New Investigator Award; Grant 99-35103-8570; $100,000; 2 Years

Assessment of damage in recent severe earthquakes has demonstrated that there is a clear and pressing need to develop reliable and economical technologies for improving the seismic performance of woodframe and timber structures. The seismic response of timber structures is predominately controlled by their connections, and when they are loaded to failure, damage is concentrated at the joints. Response of timber structures is also highly dependent on their energy dissipation capacity.

Slotted Bolted Connections (SBCs) are simple friction dampers which provide an effective and economical means for controlled, non-destructive energy dissipation in structures subjected to severe seismic loading. Results from proof-of-concept experiments and extensive research on SBCs in steel systems suggest that the potential performance of SBCs in timber joints is very promising. It is believed that SBC friction dampers will prove to be a highly effective means for damage control and energy dissipation in timber structures. SBCs are simple and inexpensive, and lend themselves to a wide range of applications (including retrofit of existing structures.) Use of SBCs will likely lead to safer and better performing wood buildings in areas of high seismic risk.

Experimental work is proposed to test twenty component SBC friction dampers and a single full-scale timber frame with a damped diagonal brace. Damping characteristics and overall performance of tested systems will be analyzed, and numerical models of SBC systems will be developed.

9902450 Load-Sharing and System Factors for Light-Frame Walls

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Strengthening Award; Grant 99-35103-8543; $120,000; 2 Years

The wood industry is facing unprecedented competition from other structural materials in the light-frame construction market in the United States. Clearly the primary framing material is dimension lumber, and while advances in the production and use of engineered wood composites is certain to continue, conventional construction using dimension lumber will remain the most widely used for light-frame construction. One promising mechanism for increasing the competitiveness of wood as a structural material, and more specifically, as a material for engineered construction, is the use of repetitive-use (or system) factors in design. These factors take into account the fact that individual structural members are actually performing as part of a structural system, with inherent load-sharing. The proposed research seeks to address the need for improved design procedures that account, in a rational and defensible way, for load-sharing and load-distribution effects in conventional light-frame walls. This, in turn, will serve to increase the competitiveness of wood as a structural material and ensure its continued use in the light-frame market.

9902352 Self-assembling Lignin-Carbohydrate Copolymers

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Grant 99-35504-8012; $175,000; 3 Years

The use of natural resources containing cellulose (wood, agricultural harvesting residues, etc.) for structural materiels increasingly requires reconstitution. Wood-like reconstituted materials have long been produced from wood components that are much smaller in dimension than the final product. Plywood, particle board, fiberboards all are examples. In wood reconstitution, the adhesion of the matrix (glue) to the surface of the particle or fiber is critical: it determines how well the composite will perform in use. Although this interfacial adhesion is the limiting aspect of all manmade composites, nature's composites do not suffer from this weakness. This can universally be attributed to the gradual nature of the transition in chemical makeup between the core of the reinforcing fiber and the interfibrillar matrix. This transitional region may extend from the mono-molecular layer to the millimeter-scale.

The design of such a gradual transition region on the surface of cellulose fibers is the goal of the present proposal. Using the molecular architecture of the chemical substances found in the interfacial region of wood as model substrates, a layered structure on wood fibers is targeted that represents the transition from a cellulose-rich fiber interior to a lignin-rich matrix. This layering will be attempted by a process known as self-assembly, whereby molecules associate with each other spontaneously based on their chemical composition.

The results are expected to further advance the replacement of large dimension timber products by wood-like composites from small-dimension natural resources (crop residues or short-rotation trees).

9902607 Biaryl Coupling in Ellagitannin Biosynthesis

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Grant 99-35103-8070; $110,000; 2 Years

Almost all of the economically important hardwoods found in the United States produce a class of extractives called ellagitannins. These compounds are most often found in the heartwood, or interior region of older trees, with the red and white oaks having the highest ellagitannin levels (up to 10% by weight). Due to their high reactivity, ellagitannins are detrimental to many wood processing operations, where discoloration and staining can lead to economic losses of 30-50%. A better understanding of ellagitannin formation can ultimately provide insight into methods that control their formation. At present, the biochemistry of ellagitannin and heartwood formation in trees remains one of the most poorly understood metabolic processes found in woody plants. The main objective of this work is to determine how the initial process in ellagitannin biosynthesis (biaryl coupling) occurs and what enzymes are responsible for the process. We have developed a series of laboratory-based cell lines of white oak, sweetgum and American chestnut, all species that produce these polyphenols in their natural environment. Using these cell culture systems, we plan to develop methods to initiate and quantify ellagitannin production and subsequently identify/characterize the enzymes responsible for biaryl coupling.

9902433 The Molecular Basis of Heartwood Formation: A New Frontier to Exploit Wood Properties

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Grant 99-35103-8037; $215,000; 3 Years.

The merchantable bole of all commercial woods primarily consists of heartwood (~95%), and this raw material serves as the starting point from which all solid wood (lumber, furniture, etc.) and pulp/paper products are derived. To a large part, desirable heartwood properties (color, texture, durability) in any particular species are imparted by the deposition of its often unique non-structural infused constituents; these in turn, help"hallmark" a particular woody species from that of others. Such infused substances, whose amounts can vary extensively between different species, are laid down in (prelignified) sapwood at some undetermined time-point in each woody plant's development. These substances, however, significantly dictate the characteristic properties of individual woods, e.g., the black color of ebony; the reddishness of cherry, and the orangish-red hue of western red cedar. These same substances also help define other characteristic traits of individual woods, such as texture and durability. The general mechanisms associated with heartwood formation are essentially unknown. This is all the more remarkable given the overall importance to woody plant growth and development, as well as in rationally designing desirable properties in selected wood types through future biotechnological manipulations. This particular project, therefore, aims to identify the overall biochemical and molecular mechanisms involved in heartwood generation, *via* defining how, when and where monolignols (and their derivatives) are differentially partitioned into the oligomeric lignan infusions of western red cedar heartwood, and into its structural lignins, respectively, at the metabolite, gene and enzyme level. In turn, this will enable us to help define the overall mechanisms in heartwood formation, and to design novel strategies for its biotechnological manipulation.

9902604 Fasteners as Diagnostic Sensors in Wood Structures

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Grant 99-35103-8038; $140,000; 3 Years

Historical damage surveys in the aftermath of hurricanes and earthquakes indicate that structural failures often initiate from, or propagate through, connections. Such damage frequently results in regulatory restrictions on the use of wood components, thus jeopardizing the utilization of wood products for structural applications. This project involves the development of a comprehensive ultrasonic inspection technique that employs bolts and nails as diagnostic sensors in wood structures. Ultrasonic pulse-echo detection of fastener deformation within connections will be utilized to measure connection displacement due to applied load, as well as energy dissipation within the connection. Overload magnitude (load beyond the yield point), internal connection damage and residual capacity will also be assessed based on the results of ultrasonic inspection of deformed fasteners. This research will lead to an improved understanding of connection failure mechanisms, an enhanced ability to model the behavior of structural wood assemblies, and a more complete understanding of systems response to static and dynamic forces. These will eventually result in more accurate modeling and design of structural wood systems, thus minimizing unsatisfactory performance in catastrophic events such as hurricanes and earthquakes. This will also help protect existing markets for structural wood products, and may enhance the use of structural wood systems in high-demand scenarios that are currently closed to wood construction.

9902446 Hardened-Steel Dowel Connections in Solid Timber and Lumber Products

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Grant 99-35103-8678; $90,000; 2 Years.

This project will evaluate the behavior of small diameter hardened-steel fasteners in solid timber and common manufactured lumber products. The project's primary focus will be on timber rivets, which have been used in Canada for over 20 years and were recently adopted into the United States timber building code. Known or anticipated benefits of these connections over nailed connections are increased stiffness and strength; benefits over bolted connections are reduced overall connection size and decreased fabrication cost. Most of the data on timber rivet behavior are for Canadian wood species. An extensive testing program, evaluating the performance of these fasteners in common United States wood species and manufactured lumber products, will form the backbone of the study. Tests on small connections will produce information about the behavior of the fasteners themselves; tests on large connections (to 120 rivets) will permit study of the crossover in failure mode from fastener yield to wood fracture. Existing analytical models and code-sanctioned limit states design procedures will be evaluated as predictors of the test data, and new models will be developed if necessary. The project will also evaluate the potential for using fasteners with extensive applications in non-wood construction, namely ballistic-point pneumatic or power-driven fasteners, as timber fasteners. The ultimate product of the project will be recommendations for design guidelines in the form of new procedures or adjustments to current design procedures.

9902440 Load Distribution in Metal-Clad Wood-Frame Diaphragms

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Grant 99-35103-8240; $143,000; 4 Years

When wood panels, metal cladding, gypsum wallboard and other sheathing materials are added to the structural frame of a building, large wall, ceiling, and roof "plates" are formed. When a ceiling or roof "plate" adds measurable strength and/or stiffness to the building, it is referred to as a structural diaphragm. When a wall adds measurable strength and/or stiffness to the building, it is referred to as a shearwall. Because of their impact on overall building design, it is important for engineers to accurately account for the behavior of all structural diaphragms and shearwalls. In the United States, the vast majority of agricultural buildings are wood frame structures that are sheathed with corrugated metal cladding. The attachment of this metal cladding has been shown to produce diaphragms and shearwalls that significantly impact building behavior. Unfortunately, engineers have not been able to accurately predict the behavior (under load) of many larger metal-clad wood-frame (MCWF) buildings (i.e., horizontal eave movements of a 1/2 foot due to design wind loads are not uncommon in buildings with high length-to-width ratios). This is largely attributable to a relatively poor understanding of the sensitivity of load distribution in diaphragms to changes in the many factors controlling load distribution. In the proposed research, a 40- by 200-foot MCWF building and several large MCWF diaphragms will be constructed and extensively instrumented. The information obtained from these tests will be used to develop design procedures that will produce safer, less expensive, and more functional MCWF buildings.

9902351 "Lignins" in Lignin-Biosynthetic-Pathway Mutants and Transgenics?

Ralph, J.

USDA Agricultural Research Service; US Dairy Forage Research Center; Madison, WI 53706-1108
Grant 99-35103-8602; $172,000; 2 Years

Lignin is a polymer that plants use to bind the fibers together and confer structural rigidity to stems as well as provide other functions for the well-being of the plant. However, utilization of plant resources is often limited by the difficulty of dealing with lignin. It is the polymer that must be removed to make fine paper, for example. Recently, natural mutant and transgenic plants in which the lignin biosynthetic pathway has been affected have become available. Such plants provide a rich source of insight into the chemistry of lignin formation that will allow more efficient uses of our plant resources in the future. Recent work on a few mutant plants revealed that deprivation of a plant's normal ability to produce lignin precursors results in increased incorporation of other plant phenols into the lignin (or lignin-like) polymer. Some of the incorporated monomers were unexpected and not associated with the normal biosynthetic pathway, although all are found at low levels in normal plants. The large compositional shifts that are possible indicates considerable plasticity in the lignification process, suggesting new approaches to plant modification for improved utilization in processes ranging from polysaccharide digestion in ruminants to industrial chemical pulping. Detailed characterization of the lignin structure in a variety of mutant and transgenic plants (in which the various lignin biosynthetic pathway enzymes have been down- or up-regulated) will be carried out here to provide further insight into plant processes and to target new ways of altering lignin for improved utilization of plant fibers.

AGRICULTURAL SYSTEMS

Panel Manager - Dr. Lloyd T. Wilson, Texas A&M University
Program Director - Dr. Timothy C. Strickland

Although agricultural research has most often focused on individual system elements, the Agricultural Systems program provides opportunities for integration of these elements through a whole systems research approach. The objective is to obtain knowledge that is essential to sustain the viability of the agricultural enterprise. Such research is needed to address directly interactions among the elements that comprise agricultural systems. It is expected that projects will include the active participation of the users of agricultural research, both in project design, implementation, and the adoption of newly developed practices and policies.

This program supports systems research that has the potential to aid in the development and/or evaluation of national, regional, community, and/or farm level practices and policies that will sustain: a safe and adequate supply of agricultural products and services; environmental quality and the natural resource base; human health; the economic viability and quality of life of rural communities; and that will improve our management of issues at the urban and rural interface.

9903391 Integrated Assessment of Sustainable Poultry Waste Management Systems
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University of Arkansas; Center of Excellence for Poultry Science; Fayetteville, AR 72701
Strengthening Award; Grant 99-35314-8655; $250,000; 3 Years

The states of Arkansas, Oklahoma, and Missouri account for 21 and 17% of the national broiler and turkey production. A by-product of intensified production is poultry litter, which contains significant amounts of nitrogen and phosphorus, making it an important source of crop nutrients. Concentrated poultry production may result in litter applications that exceed crop nutrient requirements and result in potentially excessive nutrient loadings to surface and groundwater. In 1997, the Oklahoma Conservation Commission released a report on the water quality of Lakes Eucha and Spavinaw (E/S). The report concluded that eutrophication in Lake Eucha was caused by elevated phosphorus loadings "from non-point source pollution resulting from agricultural practices associated with the poultry industry." In order to address this finding, it is necessary to identify the dominant sources of nutrients, assess the controlling transport mechanisms, identify precise economic relationships among agricultural system components, propose management and policy strategies to reduce nutrient movement in the environment and assess the impacts of implementing these strategies. The objectives of this research are: 1) analyze the existing phosphorus-flow system in the E/S watershed; 2) identify alternative phosphorus management policies which may be employed; and 3) evaluate alternative phosphorus management policies in the watershed. A phosphorus model will be tested to evaluate the model's ability to predict trends in soil phosphorus levels in pastures receiving poultry litter. Phosphorus management alternatives will be jointly fashioned by the research team and concerned parties from the E/S watershed. The eventual goal of these activities is to cooperatively establish an acceptable direction for sustainable phosphorus management.

99003388 Polyculture System Seaweed, Shrimp and Fish at Small Farm Units on Molokai, Hawaii
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Grant 99-35209-8560; $185,965; 3 Years

As on other Pacific islands, the rural community on Molokai is struggling to develop new agricultural systems to allow residents to make a living from the land and sea in the modern economy. Coastal aquaculture systems producing native fish, edible seaweeds and shrimp have been installed along the island's extensive south reef. These small farm enterprises tend to be economically unstable, and have difficulty competing with larger operations elsewhere for off-island markets. Shrimp and fish cultures also are a source of pollution to the clear-water, coral reef environment. This project will integrate fish and shrimp production with seaweed crops to recycle nutrients within each farm unit and provide multiple cash crops to stabilize the economics of production. The agricultural system will grow the seaweed, *Gracilaria parvispora*, in floating cage cultures with moi fish in traditional Hawaiian fishponds. The seaweed, known locally as long ogo, is the most valuable edible seaweed in Honolulu markets, while moi are a prized local fish for which an aquaculture production system is available. The system will also grow seaweed in effluent ditches of existing shrimp farms on the island. We will determine the ratio of seaweed to fish or shrimp required to recycle nitrogen and phosphorous released from the animal cultures, optimize the production system for each component, and develop an enterprise budget for the polyculture systems. This agricultural system can serve as a model for the United States-affiliated Pacific Island states, which are undergoing a transition from subsistence to cash crop economics.

9903385 Agriculture in the Internet Economy
Kenney, M.
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Grant 99-35209-8130; $150,000; 2 Years

In agriculture, as in many other industrial sectors, the internet is rapidly being adopted for use by all the segments of the value chain. This study proposes to measure the impacts of internet development on the structure of the U.S. agricultural system, the configuration of agricultural value chains, and the impacts on farm operators. The project addresses the following research questions: (1) How is the internet used as a tool in farm management? (2) How has the internet facilitated access to information that is critical for successful farm management? (3) To what extent do farm operators utilize e-commerce for acquiring intermediate inputs and for what agricultural commodities is the internet being used as a means for marketing? (4) How is the development of WWW changing the U.S. agricultural system? (5) How are firms in the agricultural input sector utilizing the internet to provide information and service? (6) How are firms in the marketing and agricultural processing sector of the agricultural system utilizing the internet to provide information and services to customers? These research questions will be addressed through a research design that involves (a) a sample survey assessing the use of the internet by farm operators and ranchers located in a five state region in the Midwest; and (b) initial steps to prepare interviews with internet web-site operators in the agricultural input and processing sectors.

9903473 Integrated Evaluations of Minimum Disturbance Production Systems in California's San Joaquin Valley

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Grant 99-35314-8654; $80,000; 3 Years

Despite a 300% increase in conservation tillage (CT) production in the Midwest during the past decade, less than 0.3% of the acreage in California's San Joaquin Valley is currently farmed using CT practices. Preplant tillage operations typically account for 18-24% of overall production costs for annual crops grown in the West Side region of the San JoaquinValley (SVJ). An average of about 9 to 11 tillage-related passes are routinely done during the fall-spring period to *prepare* the soil for summer cropping. These passes represent not only considerable energy, equipment and labor costs, but recent research indicates that tillage reduces soil organic matter (SOM) and emits considerable respirable dust as well. Because SOM is widely regarded as an important attribute of good soil quality and long-term productivity, interest has been growing over the several years, in developing alternative production systems that reduce costs while at the same time improve the soil resource through greater carbon sequestration. Furthermore, no data exist on potential benefits of reduced tillage production systems in terms of mitigating airborne respirable dusts that are known to result from heavily tilled agricultural production systems. Conservation tillage systems may serve to increase SOM levels, reduce production costs and improve air quality in this critically important agricultural production. The research project proposed here will compare conservation tillage and conventional tillage practices in crop rotations common to the SVJ's West Side in terms of farm productivity and profitability, key soil quality indicator properties, pest and crop management requirements, and the potential for improving air quality, and will widely disseminate information related to the background, goals and outcomes of the proposed study.

9903441 A Year Round Forage Production Manure Utilization System
Newton, G.L.; Vellidis, G.; Gascho, G.J.
University of Georgia; National Environmentally Sound Production Agriculture Laboratory (NESPAL); Tifton, GA 31793-0748
Grant 96-35211-3866; $270,000; 2 Years

The goals of the project are to develop, evaluate and demonstrate an economically and environmentally sound method of manure utilization and crop production within a high-yield, intensive agricultural system. The research will use both replicated plots and a field scale center pivot irrigation system, two minimum tillage year round cropping sequences, and a riparian wetland to determine: 1) crop nutrient removal, use efficiency, yield and digestibility associated with manure or commercial fertilizer application; 2) the fate and transport of nutrients in surface runoff, soil and ground water associated with liquid manure and commercial fertilizer applications, and the environmental value of an adjacent riparian wetland; 3) the response of nematode and insect populations to application of liquid dairy manure on year round forage production systems and the effects of nematodes on nutrient movement; and 4) the economic consequences of replacing commercial fertilizer with irrigated liquid dairy manure for year round forage production, the economic value of a riparian wetland, quantify economic/environmental tradeoffs, and the economics of year round manure-forage production as compared with less intensive practices. Crop responses, soil processes, unsaturated zone water quality and movement, and ground and runoff water quality and movement, including fecal coliforms in runoff, will be monitored. A model will be developed to predict the economic consequences of manure applications, including restricted phosphorus inputs. Results of the project will allow farmers to continue to satisfy human needs, enhance the long-term viability of food production, improve productivity and improve or maintain environmental water quality.

9903459 Systems Assessment of High-Oil Corn at the Farm, Elevator and End-User Levels
Dooley, F.J.; Fulton, J.R., Maier, D.E.
Purdue University; Department of Agricultural Economics; West Lafayette, IN 47907-1145
Grant 99-35211-8018; $260,000; 4 Years

The introduction of value-added grains, such as high oil corn (HOC), may radically alter the present grain marketing system. Farmers, elevators, and end-users face new opportunities as they consider producing, handling, or utilizing value-added grains. However, levels and types of risk are uncertain for most parties. Furthermore, the appropriate market mechanisms to govern these new arrangements are unknown, and the distribution of value remains unclear.

The proposed research seeks to answer two basic questions. First, what is the value of high-oil corn? Second, how is the value of HOC allocated among the participants in the grain marketing system? These questions are asked in the context of alternative market coordination systems, production and marketing uncertainty, and the interactions among system participants. This analysis will develop a system dynamics modeling framework for analyzing the impacts of value-added grains on participants in the market coordination system. As such, the model can generate results that provide information on the most likely marketing system developments and distributions of premiums for value-added grains.

9903475 Ecosystem Management in a Landscape Dominated by Small Private Ownerships

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University of Massachusetts, Amherst; Department of Forestry and Wildlife Management; Amherst, MA 01003-4210
Grant 99-35108-8046; $160,000; 2 Years

Nationally, 57% of forestland is owned by non-industrial private individuals. In some regions of the country, such as the northeast, as much as 85% of the forested landscape is in this ownership. Recent advances in forest ecosystem research have identified the important reasons why an ecosystem-based approach to forest management is advantageous (e.g., forest health, protection of ecosystem functions, maintenance of biodiversity). The nation's forest will be better able to meet societal goals if they are managed with this sensitivity at the ecosystem level. If an ecosystem-based approach is to be adopted in many parts of the nation, it must be embraced by non-industrial private owners. This proposed research will weave together landowner attitudes with new concepts of ecosystem management. The result will be an improved understanding of how this new paradigm might be successfully applied.

Specifically we propose to follow-up on our pilot study of landowner attitudes that identified landowner agreement with certain ecosystem-based management principles. We will use a similar mail survey instrument with a broader population of landowners to test the wider applicability of our results. In addition, we will use geographic information system technology in an interactive focus group setting to further test landowner attitudes towards cooperation across boundaries and management at a broader ecosystem scale. As the basis of the interactive GIS approach, we will use a model property tax assessment program which affords tax relief to those landowners that take a more ecosystem-based approach to their land and its management and protection.

9903379 Improved Information in Support of a National Strategy for Open Land Policies

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Strengthening Award; Grant 99-35403-8658; $155,072; 2 Years

Nearly 200 state and local initiatives to curb sprawling development were on a November 1998 ballot (Washington Post). The Federal Agricultural Improvement and Reform Act of 1996 (FAIR) authorized up to $35 million (from Commodity Credit Corporation funds) for state and local farmland protection programs. More recently, Vice President Gore announced a $9.5 billion bond proposal to curb urban sprawl and proposed $1 billion in tax credits and grants to help communities preserve farmland (Gore). Given this broad perspective of open land protection, it is striking that the policy debates are missing reliable and consistent information about what, exactly, supporters of such programs really care about and value. This study is designed to identify and quantify public preferences for the provision of open land through agricultural lands, and will investigate how the quality of open land is affected by the agricultural system employed. The design provides policy relevant information regarding what attributes open land provided by agricultural lands the public prefers, the effects of the agricultural systems on the quality of open land and regional differences in public preferences.

The research proposed here will use qualitative research methods. The qualitative research will involve focus groups held in five locations throughout the U.S. The quantitative component will involve a mail survey that includes conjoint questions designed to learn what attributes of agricultural lands are important in providing open land and the economic value the public places on open land provided by agricultural lands. The integration of the results from the focus groups and mail surveys with a review of existing open land protection efforts will enable us to make design recommendations for the future programs, policies and legislation to protect open land through agricultural lands. This integration will enable us to design policies and legislation so that public preferences will be addressed and the proposals will gain greater public support. This policy analysis will also make recommendations regarding what level of government should be involved in protection efforts, whether different levels of government should be involved in different issues relating to protection of agricultural lands for open land, and whether efforts should involve joint public, nonprofit, and private efforts. Finally, we will address how policies that effect agricultural systems can be developed to enhance the role of agricultural lands in the provision of open land.

9903460 An Intelligent Encyclopedia of Oak Cover Type Ecosystems in Southern Appalachia

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Grant 99-35108-8553; $210,000; 3 Years

Many social and economic institutions in the southern Appalachians depend on benefits afforded by upland oak forests. Traditional rural economies rely on abundant, high-quality timber; plentiful, diverse wildlife and fish; extensive recreational opportunities; and several special forest products. Public and private managers of these ecosystems are challenged by an increasingly complex set of benefits, but continue to rely on traditional *ad hoc* management practices. A gap remains between what scientists have learned about sustainable upland oak ecosystems here and what land managers can apply. Currently, an overwhelming body of information about these ecosystems is not easily accessible nor readily useable

because it has not been synthesized and integrated into a coherent, meaningful knowledge structure that is useful for problem solving. The primary objective of the proposed project is to synthesize and integrate the past 65 years of southern Appalachian research on upland oak ecosystems, and to translate it into an "intelligent," hyperdocument-based encyclopedia system that is accessible over the internet. We propose to identify pertinent research knowledge, identify its content and structure (links), and codify that content and structure into hypertext format. More than a Web site, the encyclopedia will incorporate programmed "intelligence" in knowledge-based systems and simulation models for problem solving and decision support and will integrate database access. It will be dynamic, making future updates easier, and nonlinear, allowing a greater level of knowledge integration than print media can accommodate.

9903394 Strategies for Transition to Organic Systems: Ecological and Economic Indices.

Creamer, N.G.; Mueller, J.P.; Wagger, M.G.; Barbercheck, M.E.; Louws, F.J.; Brownie, C.; Marra, M.C.; Monks, D.W.; Wossing, A.; Koenning, S.
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Grant 99-35108-8101; $380,000; 4 Years

This study will investigate various strategies, from a biological and economic systems perspective, for making the transition from a conventional to an organic agricultural production system. It has been documented that when growers transition from conventional to organic production systems, there is a period of suppressed yields followed by a return to yields near or equal to conventional production. This "transition effect" has been attributed in part to time required for necessary changes in chemical, physical, and biological properties of soil which enhance nutrient cycling, plant growth and development, and the biological pest control properties of the system. When making this transition, the producer has a choice to affect the change rapidly and completely on all or part of his/her farm, or gradually over a period of a few years by progressively exchanging inputs from the conventional system for plant and animal manures, longer rotations, cultivation and mulching, beneficial organisms, etc. Six treatments, representing various strategies for transition and appropriate controls, will be monitored for energy and nutrient flows within the plant-soil system. Soil quality indices, including a range of physical, chemical, and microbiological properties as well as micro and macro fauna, weed, disease and insect dynamics, and overall productivity and economic performance will also be monitored. Finding ways for farmers to switch to environmentally sensitive management practices and at the same time to high value crops like organic commodities, may give them the needed income that allows them to continue to stay in business.

9903472 Integration of Remote Sensing, GIS and Soil Physics for Disease Detection and Management

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Grant 99-35108-8113; $173,000; 3 Years

Phytophthora species are responsible for root and crown diseases of many horticultural crops. Under the most severe (acute) conditions these diseases can lead to complete crop loss, while under less severe (chronic) conditions the losses are due to the effects of a diminished root mass. Very little research has been conducted on the chronic effects of *Phytophthora* on crop productivity because of the inherent difficulties in detection. The development of root rot is directly related to the drainage characteristics of the soil. In this research, the relationship of soil drainage and soil physics with expression of both chronic and acute injury will be investigated. The approach will include standard methods for crop, soil and pathogen evaluation, and these data will be combined in a GIS database. A remote sensing component will include evaluation of aerial photography for crop damage assessment and specifically, detection of chronic injury by *Phytophthora*. The results of this research will be vital in quantifying the chronic impacts of *Phytophthora* root rot on crop yield, determining the soil factors (especially drainage characteristics of soil) that enhance chronic infections, and finally development of a method for detection and spatial description of the *Phytophthora* affected areas. The results of this research could empower practical growers with new tools for efficiently implementing cultural practices such as soil management to improve crop yields.

9903397 Developing Environmentally Sustainable and Economically Viable Cropping Systems

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Grant 99-35108-8005; $225,000; 3 Years

Approximately three million acres of soybeans, corn and wheat are grown in the Mid- Atlantic region (MD, NC, PA, and VA) and are a significant factor in the rural economy ($1.5 billion in 1995). Soybean and corn yields on low water-holding capacity soils in the region are inconsistent, often below national levels, and are unprofitable in many cases. Research is needed to develop practices that improve the profitability of the standard corn, wheat and double soybean rotation. Alternative cropping systems also need to be evaluated to ascertain the most environmentally sustainable and economically viable system for this region. The overall goal of this research is to enhance grain yields and farm profits with

environmental protection. Specific research goals are to increase precipitation use efficiencies, improve soil quality, and optimize nutrient loading into the soil system. The project will evaluate the standard corn, wheat, double crop soybean rotation with two other rotations, one more intensive (4 crops in 2 years), and one less intensive (4 crops in three years). Modern commercial farm equipment equipped with GPS, yield monitors and computer controllers for viable rate treatments (seed and nutrients) are being used. The experiment is laid out in strips 60 feet by 2000 feet long (3 acres), with seven strips per replication, in order to have each cropping system present each year. A detailed technology transfer plan has been developed to transfer research findings to growers, agribusiness personnel, extension agents, NRCS and other research groups.

9903392 Integrating Nutrient Management, Modeling for Dairy Production Systems Featuring Dynamics and Optimization

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Washington State University; Biological Systems Engineering Department; Pullman, WA 99164
Grant 99-35209-8573; $340,000, 3 Years

A sustainable dairy industry depends upon the development of best nutrient management practices that maintain profitability of a farm operation while minimizing environmental degradation. The goal of this project is to develop a multi-objective decision support system (MODSS) based on a systems approach for nutrient management that considers animal, crop, waste, and soil subsystems in a whole dairy farm. The goal will be accomplished by integrating existing models that address individual aspects of a dairy operation with economic optimization and multi-objective decision-making techniques. The project will also produce monographs that illustrate the relationship between the major decision variables. Different applications of the MODSS can serve government agencies, researchers, and consultants for their intended specific uses. The monographs can also be used by extension personnel and by farmers. The unique aspects of this project include the use of a systems approach for dairy system modeling, the consideration of the dynamics of nutrient flow, and the interface between process modeling and optimization techniques. Main applications of the research products include evaluating alternatives and optimizing management decisions at the farm level under given economic and environmental constraints. The completion of the project will provide an effective tool to the dairy industry for enhancing its sustainable development.

9903471 Sustainability of Three Apple Production Systems

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Grant 96-35314-3897; $185,000; 3 Years

We established in 1994 a 1.7-hectare replicated study of organic, conventional, and integrated apple production systems in a new commercial 'Golden Delicious' orchard in Washington state. Our project is unique in that it is an on-farm, grower/scientist-managed study. In the first five years of our study, we have already found significant differences in soil quality, crop quality, and financial performance among these systems. We intend for our study to run at least 10 years and are requesting funding for the next three years (1999 to 2002).

Our goals are to determine how system components are integrated within organic, conventional, and integrated apple production systems and to provide a full range of management options to apple growers. Supporting objectives include measuring system components: soil quality, crop quality, pest interactions, and financial performance. A final supporting objective is to develop a whole farm sustainability index for each of our three farming systems.

Experience gained from our study will contribute to improvements in the production practices of organic and integrated apple growers and in the ability of conventional apple growers to convert to organic production methods or adopt integrated management approaches. Information that our study adds to the knowledge base of alternative apple production systems will expand economic opportunities for farmers and promote reduced use of agrochemicals. Results from our study will be disseminated to cooperative extension agents, growers, professional horticulturists, students, and the scientific community. Benefits from our study will contribute to enhanced sustainability of fruit production systems in the U.S.

9903479 Transition Methods To Convert from Conventional To Organic Agricultural Systems

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University of West Virginia; Division of Plant and Soil Sciences; Morgantown, West VA 26506
Grant 99-35108-8227; $103,329; 3 Years

Organic farming and gardening have the potential to be sustainable agricultural systems. In addition, they address issues of food safety and environmental pollution. Demand for organically produced foods has increased by 20% per year since 1991, and future demand appears assured. However, up to 3 years are required to make transition from conventional production to a certified organic system. Net farm income may drop during this transition period and producers may be reluctant to make the change due to short-term risks involved. Research proposed here will compare two farming systems for making the transition from conventional to organic production. Replicated experiments in both a market garden and a field crop/livestock farm will be used to compare a low-input cover cropping system with a high-input compost amendment

system over a 3 year period. The crop/livestock farm will also compare presence and absence of animals in the system. Criteria for evaluating systems will be crop and animal productivity; soil quality enhancement, as measured by physical, chemical and biological factors; pest populations; and analysis of labor and management inputs to estimate profitability on a system basis. Additional component experiments will evaluate compost amendment rates and weed management practices in more detailed small-plot trials. These data will provide those growers interested in converting to organic practices with a reliable basis for choosing among transition practices in order to overcome traditional barriers to transition while maximizing productivity and sustainability of their production systems.

9903377 The Effects of Organic Manures and Biodynamic Growth Regulators on an Alternative Cropping System

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University of Wisconsin; Michael Fields Agricultural Institute; Center of Integrated Agricultural Systems; Madison, WI 53706

Grant 99-35211-8064; $380,000; 3 Years

The rotation of maize-soybeans-winter wheat + red clover has potential as an alternative to maize soybean systems. It should be profitable, will significantly reduce the use of agrochemicals and offer increased management options for medium and small farms. However, questions remain concerning the stability of maize yields with this expanded rotation. Based on previous research we think that: 1) the positive effects of rotation and manure are due to improvements in the efficiencies of rooting systems; 2) the crop yields from the system depend on soil having adequate supplies of biologically active organic matter; 3) combining the system with manure or compost inputs will lead to improved yields and greater profitability; and 4) using biodynamic growth regulators will increase rooting, the production of active organic matter and the stability of yields. To test these ideas we will explore linkages between the use of crop rotations, compost, manure, and biodynamic regulators on the one hand and the formation of active organic matter, the dynamics and efficiency of root activity, mycorrhizal infection, and the nutrient uptake and yield maize on the other. The study will involve two field experiments on different sites. Both experiments will compare alternative systems to commonly utilized, conventionally managed systems. Miniplot studies will clarify ideas on the mode of action of manure and compost increasing active organic matter. The results of our research will feed into ongoing implementation projects and will also be used to test an organic matter budgetor that is designed to help farmers plan sustainable options. The project addresses the economic viability of family farms, their long-term productivity, and the enhancement of the natural resource base. It is expected to enhance sustainability and to provide a model for wider application in agriculture.

STRENGTHENING AWARDS

Program Directors: Mr. Jeffery L. Conrad, Dr. Liang-Shiou Lin,
Dr. Carl Profater, and Dr. Deborah Sheely

Strengthening Awards are intended to enhance the ability of faculty of small and mid-sized institutions or of institutions in USDA-EPSCoR States who have not previously been successful in obtaining competitive grants from the NRICGP in receiving support for strengthening their research programs. The research must be in an area supported by the NRICGP. The following types of Strengthening Awards are available: Research Career Enhancement Awards (to fund sabbatical leaves); Equipment Grants (to supply 50% of the funds necessary to purchase one major piece of equipment within the cost range of $10,000-$250,000); Seed Grants (to collect preliminary data); and Strengthening Standard Research Project Grants (to support standard research projects). Applications for the Research Career Enhancement Awards, Equipment Grants and Seed Grants were reviewed together by multi-disciplinary peer panels. Strengthening Standard Research Project applications were reviewed in the program area most appropriate for the type of research proposed; therefore, these nontechnical abstracts also appear under the respective program areas.

9900941 The Role of Organic Nitrogen in the Nitrogen Economy of Taiga Trees

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Strengthening Award; Grant 99-35101-7859; $467,500; 3 Years

Contrary to conventional wisdom, the conversion of organic nitrogen to inorganic forms is not a prerequisite for plant uptake. Plants from a variety of ecosystems, including several important agricultural species, have been shown to have the capacity to absorb organic nitrogen directly. In the context of low-input sustainable agriculture and forestry, these findings are extremely important. Here we propose to investigate if organic nitrogen plays a significant role in the nitrogen economy of taiga ecosystems. The research is based on four important observations: First, data for ecosystem nitrogen budgets in taiga forests show that the annual plant nitrogen requirements significantly exceed the annual inorganic nitrogen flux. Second, some taiga species have been shown to absorb organic nitrogen under field conditions. Third, taiga soils have large concentrations of soluble organic nitrogen. Fourth, inter-specific variation in the stable isotope chemistry of taiga trees suggests that plants may derive nitrogen from several different sources, including organic forms. The plan calls for a combination of intensive and extensive studies across primary (floodplain) and secondary (upland) successional sequences in the Alaskan taiga. The research will include: (1) uptake of organic nitrogen in the field, (2) determination of species-specific variation in $\delta^{15}N$ signatures, (3) turnover studies of organic and inorganic soil nitrogen fractions, and (4) ecosystem nitrogen budget determinations. The proposed research should advance our knowledge regarding the fundamental mechanisms of nitrogen cycling in taiga forests, and has profound ramifications for both global change research on natural ecosystems and applied agricultural practices.

9903670 Acquisition of an Ultramicrotome

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Equipment Grant; Grant 99-35208-8582; $16,384; 1 Year

The Auburn University Biological Electron Microscopy Imaging Facility (BEMIF) is a multi-disciplinary, research instrumentation, service facility that is used extensively for research in the biological sciences, agriculture, engineering, textiles, soils, geology, and material sciences. Acquisition of a USDA-NRICGP supported state-of-the-art ultramicrotome will strengthen BEMIFs ability to perform currently funded research and to develop new research areas utilizing modern ultrastructural techniques. This new instrument will provide for a significant increase in BEMIF's available ultramicrotomy and TEM services, thus allowing for the use of a much wider range of advanced research techniques, as well as new instructional opportunities. Several projects in collaboration with BEMIF are related to agriculture and are focused on expanding our basic understanding of numerous biological systems. A study that will benefit from the newly acquired ultramicrotome is aimed at increasing our understanding of genetically engineered cotton fibers. This project is currently funded by the USDA and uses the following strategies: 1) create recombinant DNA for enhanced protein expression in cotton fibers; 2) transform cotton plants with the DNA and assay for protein expression; and 3) assay fiber qualities using microscopy and other physical testing methods. Results from this study will be used to provide value-added traits to textiles and benefit the textile industry. Another research study that uses the facilities and equipment located in BEMIF is concerned with an analysis of the cellular and molecular mechanisms involved in supporting symbiotic associations that are critical components of economically important fisheries and land based agriculture.

9903681 Acquisition of a High-Performance Liquid Chromatography System

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Equipment Grant; Grant 99-35106-8400; $34,015; 1 Year

The high-performance liquid chromatography (HPLC) system is one of the most effective and widely used tools for the analytical separation and quantification of a variety of chemical compounds. An HPLC system is requested to support primarily soil and environmental science research in two areas: 1) effects of bacterial and soil characteristics on the bioavailability of soilsorbed pesticides; and 2) microbial transformation of imidazolinone herbicides. Other ongoing research projects that will benefit from the acquisition include studies on the fate and transport of chlorinated solvents in soil and groundwater, complexation of aluminum with soil humic substances, and effects of environmental factors on methane emissions from soil amended with swine slurry. The requested HPLC system includes an autosampler, a solvent delivery system that handles up to four solvents, a diode array detector that permits scanning multiple wavelengths in one run, a flow-through radioactivity detector, and a computerized data-handling system. It will replace the 16-year old, deteriorated HPLC syst~m in the Department of Agronomy and Soils. The availability of the requested equipment will greatly enhance the facilities for research and teaching in the department and provide state-of-the-art equipment that is needed for obtaining future extramural funding.

9901479 State Transition Effects on the Mechanism of Fissure Formation in Rice Kernels

Siebenmorgen, T.J.; Bautista, R.C.; Wang, Y.-J.
University of Arkansas; Department of Food Science; Fayetteville, AR 72704
Grant 99-35503-8666; $140,000; 3 Years

The overall goal of this project is to increase the quality, value, and profitability of domestic rice production through a fundamental understanding of milling quality losses due to kernel fissures created during the drying process. Our recent research has prompted the hypothesis that fissuring occurrence is directly related to the moisture content gradient in the kernel and the resultant material properties that it exhibits based on the local state of the material. The rice kernel will go through a state transition during drying/tempering from a “glassy” state to a “rubbery” state at a moisture-dependent temperature known as the glass transition temperature. The physicomechanical properties of the kernel material change dramatically at the glass transition temperature. Although the glass transition temperature of rice can be determined by using a differential scanning calorimeter or a thermomechanical analyzer over a range of moisture contents, the dynamic thermomechanical analyzer provides more accurate and detailed information regarding the viscoclastic and transport properties of rice at different temperatures, moisture contents, and frequencies. We will use the measured diffusion and mechanical property changes resulting from state transition to explain fissure formation.

9903974 Pectins from Soy Hull
Kalapathy, U.
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Seed Grant; Grant 99-35501-8303; $48,837; 2 Years

World production of commercial pectin is estimated to be over 20,000 metric tons per year. Pectins are mostly used as gelling agent in jams and jellies and as a filler or stabilizer in confections, dairy products, fruit preparations, bakery fillings, icings and frostings. Development of pectin based fat replacer has resulted in wide range of applications in products such as spreads, salad dressings, ice cream and emulsified meat products. While numerous functional and nutritional applications of pectin exist, the only sources of commercial pectin are currently, citrus peels and apple pomace. Hence, conversion of inexpensive soy hulls into more commercially viable and marketable high value pectin products will add value for this under-utilized co-product of soy industry. Soy hull is a good source of pectin (18-20%) and we have shown that soy hull pectin could be extracted by adapting the acid extraction method used for citrus pectin production. Pectin was extracted from ground soy hull by acid extraction followed by alcohol precipitation. The current study was designed to investigate the effect of extracting acid strength, extraction temperature, and the pH of precipitating solvent on the yield and purity of pectin. Research is in progress to produce soy pectin in large scale and to compare the functional properties of soy hull pectin with citrus pectin. Research findings from these studies will lead to a competitive product from soy hull to currently commercially available citrus and apple pomace pectins.

9903391 Integrated Assessment of Sustainable Poultry Waste Management Systems
Goodwin, Jr., H.L.; Willet, K.D.; Sauer, T.J.
University of Arkansas; Center of Excellence for Poultry Science; Fayetteville, AR 72701
Strengthening Award; Grant 99-35314-8655; $250,000; 3 Years

The states of Arkansas, Oklahoma, and Missouri account for 21 and 17% of the national broiler and turkey production. A by-product of intensified production is poultry litter, which contains significant amounts of nitrogen and phosphorus, making it an important source of crop nutrients. Concentrated poultry production may result in litter applications that exceed crop nutrient requirements and result in potentially excessive nutrient loadings to surface and groundwater. In 1997, the Oklahoma Conservation Commission released a report on the water quality of Lakes Eucha and Spavinaw (E/S). The report concluded that eutrophication in Lake Eucha was caused by elevated phosphorus loadings "from non-point source pollution resulting from agricultural practices associated with the poultry industry." In order to address this finding, it is necessary to identify the dominant sources of nutrients, assess the controlling transport mechanisms, identify precise economic relationships among agricultural system components, propose management and policy strategies to reduce nutrient movement in the environment and assess the impacts of implementing these strategies. The objectives of this research are: 1) analyze the existing phosphorus-flow system in the E/S watershed; 2) identify alternative phosphorus management policies which may be employed; and 3) evaluate alternative phosphorus management policies in the watershed. A phosphorus model will be tested to evaluate the model's ability to predict trends in soil phosphorus levels in pastures receiving poultry litter. Phosphorus management alternatives will be jointly fashioned by the research team and concerned parties from the E/S watershed. The eventual goal of these activities is to cooperatively establish an acceptable direction for sustainable phosphorus management.

9903658 Near Infrared Reflectance Spectrometer For Forage Research in Southwest Arkansas

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Equipment Grant; Grant 99-35208-8511; $29,775; 1 Year

A near infrared reflectance spectrometer (NIRS) will enhance research efforts at the Southwest Research and Extension Center of the University of Arkansas. The NIRS will be used to support research aimed at improving sustainability of animal agriculture in the region through better understanding of the interaction of soils, plants, and animals in agricultural systems. The instrument works by recording near-infrared radiation reflected from samples, comparing reflectance patterns to samples of known composition, and thereby providing an estimate of carbon-containing components of samples. With development of appropriate calibration equations, this allows measurement of the nutritional quality of livestock feeds and forages (including energy, fat, protein, fiber, some minerals, and toxins) with more speed and less expense, sample size, and chemical usage compared to analysis by conventional chemistry. The instrument can also be used to determine the proportions of different species in forage mixtures or the proportions of plant parts such as leaves and stems in a fraction of the time and cost required for hand-sorting of samples. Other anticipated uses for the NIRS include screening of forage legumes for leaf and root diseases that are a major limitation to survival, analysis of soil organic carbon accumulation, analysis of factors related to efficiency of digestion by cattle, and prediction of the quality of diets consumed by grazing cattle. The NIRS will greatly increase our sample handling capability and ability to draw conclusions from a wide spectrum of measurements taken across the entire agricultural system.

9902695 Dietary Contributions to Fitness of Males of Two Invasive Tephritid Species

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Strengthening Award; Grant 99-35302-8630; $100,000; 2 Years

True fruit flies, such as the Mediterranean fruit fly and the walnut husk fly, are invasive pest species capable of inflicting crop damage costing global economies millions of dollars each year. In California, the current preventative program against Mediterranean fruit fly costs over $13 million per year. A key aspect of this preventative program is the release of sterile male Mediterranean fruit flies to interrupt the reproductive cycle of the flies. Studies have shown that both health and survival of sterile males are generally poor, likely due to the stresses of mass-rearing and irradiation. Our goal is to improve the effectiveness of the sterile insect release technique by investigating the influence of adult diet on male fruit fly performance in terms of survivorship, flight, and physiological indicators of health. We plan to study two species of fruit fly, the walnut husk fly which has successfully invaded and colonized California, and the Mediterranean fruit fly which is a constant invasive threat in California. We will investigate the potential contribution of microorganisms, including bacteria and yeast, in fruit fly diets to: 1) fly physiological performance, 2) energy expenditures for flight, and 3) the development of healthy digestive tracts. Overall fly performance and health will be evaluated biochemically, microscopically, behaviorally, and ecologically.

9902774 Irradiation in a Combination Approach to Enhance Vegetable Safety

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Strengthening Award; Grant 99-35201-8554; $128,000; 2 Years

Fresh-cut fruits and vegetables are often considered among the most healthful and safe foodstuffs, yet new disease-causing microorganisms continue to emerge and an increasing number of outbreaks are being traced to fresh produce. With the understanding that no single preservation technique will effectively destroy pathogens while maintaining quality, our objective is to determine the optimum levels of irradiation, modified atmosphere packaging (MAP), and chlorination that will enhance safety while preserving sensory attributes such as texture, color, and flavor. Selected products include shredded iceberg lettuce and shredded cabbage and other minimally processed vegetables in which the cut surfaces provide a substrate for the proliferation of spoilage and pathogenic microorganisms. Conventional sanitizing procedures are inadequate, however, harsher treatments can affect fresh-like quality. Because irradiation has the ability to penetrate inside cells, it can be highly effective to treat these products. Additionally, chlorination can destroy surface contamination while MAP can extend storage time. Together these techniques can reduce overall levels of microorganisms and hence enhance safety with the added benefit of extending shelf-life. In the absence of immediately applicable solutions, this project will reveal the efficacy of combination processing to address the emerging safety issues associated with minimally processed vegetables. The added security, as well as the economic benefit of shelf-life extension, should encourage consumers and processors to utilize this novel combination of accepted and proven technologies.

9901358 Intralocus and Intragene Recombination in the *Gli-B1* and *Glu-B3* Loci in Wheat

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Strengthening Award; Grant 99-35301-7593; $120,000; 2 Years

We will study the pattern of recombination in the multigene loci *Gli-B1* and *Glu-B3* in wheat, encoding gliadins and low molecular weight (LMW) glutenins. These two storage proteins contribute over one half of the observable variation in the technological properties of wheat. Because of very tight linkage between the two, little is known about the effects of allelic variation at each locus, their organization, or the contribution of individual genes to the properties of wheat. We will saturate the critical region of the chromosome arm lBS with at least 800 cross-overs per 1 cM produced at two levels of chromosome pairing stringency control (normal, *Phl,* and mutant, *phlb)* by using a recombination-enhancing translocation and screening for flanking markers. This should let us determine the number and the order of genes present at each locus, to break linkages between the two loci and between individual genes in each locus, and to generate new hybrid alleles. We will develop PCR methods for high resolution mapping within the loci and to produce DNA markers for individual alleles. We expect that the materials and methods developed in this study will let us define in the future the criteria of the DNA sequence similarity for crossing over as well as generate convenient DNA markers for various alleles of storage proteins for plant breeding. This study will also generate genetic stocks of wheat for field tests of the technological properties of new genetic variation.

9901488 Plant Responses to Phytophagous Insects: The Silverleaf Whitefly-Squash Interaction

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Strengthening Award; Grant 99-35301-8077; $100,000; 2 Years

The silverleaf whitefly is a severe agronomic pest and causes the developmental disorder squash leaf silvering. Squash genes *(SLW)* induced by the silverleaf whitefly have been identified and will increase our understanding of plant-defense responses to phloem-feeding insects. Additionally, if *SLW* genes are strictly correlated with the leaf slivering disorder, this research will lead to an understanding of the mechanisms that cause leaf silvering. Long-range goals are to design of cogent transgenic strategies to ameliorate the developmental disorders induced by the silverleaf whitefly and to identify the signals that induce *SLW* expression. *SLW1* encodes a M20B peptidase that is expressed in flowers and fruit and in response to whitefly feeding, jasmonic acid, ethylene, and water deficit. *SLW3* encodes a leaf-specific -glucosidase that is only induced by whitefly feeding and water deficit. *SLW3 is* regulated by a novel signaling pathway, since it does not respond to known defense/wound signals. We will determine if the levels of SLW1 and SLW3 proteins correlates with their RNA levels. Since access to substrates for these enzymes will be determined by their localization in the cell, the location of the SLW proteins will be determined. The levels of *SLW* RNAs and proteins will also be assessed in (a) silvering lines of *Cucurbita,* pea, and tomato and silvering-resistant *Cucurbita,* (b) plants that exhibit other silverleaf whitefly-induced developmental disorders, (c) *Arabidopsis* during whitefly feeding, water deficit and development. Finally, to determine the likely role of the *SLW1* M20B peptidase and the *SLW3* - glucosidase their substrates will be determined.

9900966 The Role of Cattle Grazing in Conserving Grassland Biodiversity

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Strengthening Award; Grant 99-35101-8234; $125,000; 3 Years

This research investigates the potential for using cattle to restore and manage grassland ecosystems and their associated annual wildflowers. California coastal prairie is a threatened ecosystem that evolved with disturbances such as ungulate grazing and fire; in recent history, cattle have been a dominant component of disturbance regimes in coastal prairie. Understanding the effect of cattle grazing on annual wildflowers is of particular importance because these plants comprise much of the species diversity in coastal prairie, and many are at risk of extinction. This research consists of two complementary components: a large-scale survey and manipulative experiments. First, adjacent grazed and ungrazed sites will be surveyed throughout north/central, coastal California to compare the effects of cattle grazing on annual wildflower populations over a regional scale. Second, various treatments within cattle exclosures will test the effects of different grazing regimes, thatch removal, and soil disturbance. The effects of these treatments will be studied with respect to both the extant annual wildflowers and to the seeds, seedlings, and adults of two focal species, the endangered Santa Cruz tarplant (*Holocarpha macradenia*) and bearded clover (*Trifolium barbigerum* var. *barbigerum*). This research will expand our understanding of the effects of cattle grazing on annual wildflower populations. Coastal prairie management using livestock has the unusual and promising potential for meeting short- and long-term needs for agricultural production and maintaining biological diversity.

9901032 Development of Novel OPH-based Materials for Detoxification of Organophosphate Pesticides

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Strengthening Award; Grant 99-35102-8597; $100,000; 2 Years

Synthetic organophosphorus compounds are used extensively as agricultural and domestic pesticides including insecticides, fungicides, and herbicides. These compounds are extremely toxic and act by inhibiting the nervous system of living organisms. Due to the growing environmental problems associated with these pesticides in food products and water supplies, there is a great need to develop safe, convenient, and economically-feasible methods for pesticide detoxification.

Enzymatic degradation of organophosphorus pesticides has been a subject of considerable attention during the past ten years. Organophosphorus hydrolase (OPH) isolated from soil microorganisms has been shown to degrade a wide range of organophosphate pesticides. However, the effectiveness of degradation varies among different pesticides, ranging from highly efficient with paraoxon to relatively slow with methyl parathion. This research will seek to "fine tune" and broaden the substrate specificity of OPH *via* sequential cycles of DNA shuffling and screening. The ultimate goal is to build up a collection of novel OPHs that can be rationally combined to degrade a mixture of target pesticides.

9903427 Soil Nutrients, People, Livestock and Wildlife in Rangelands of Kenya

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Sabbatical Grant; Grant 99-35106-8182; $58,460; 1 Year

Livestock and wildlife populations often compete for the same resources and there are few places where this competition is more profound than on the savannas of East Africa. It is often assumed that as domestic animal populations increase, wildlife populations decline. However, detailed data from Kenya show that on a national level there have been declines in both livestock and wildlife populations over the past 20 years. The objectives of this research project are to determine the roles of soil nutrients as indicators and facilitators of key habitats for wildlife and livestock in open-range savannas that have been used simultaneously, for millennia, by people and their livestock as well as wildlife. The key habitats are formed from a unique type of disturbance that is now absent from most wildlife protection sectors: abandoned settlement sites. Nomadic groups build village sites that are abandoned after 3 to 8 years. During occupation, the soils are enriched by manure inside the settlement sites. After abandonment, these sites become hot spots for animal activity (both wild and herded) because they become persistent, reliable sites of higher quality forage, attracting numerous wildlife species and they are probably the center-points of spatially focused animal activity. Since the removal of most human populations from National Parks, newly abandoned settlement sites have been eliminated from the very areas that are intended for highest wildlife population densities. I propose to date and sample abandoned settlement sites in Kenyan savannas. Soil samples for C, N and P analysis, horizon descriptions and soil bulk density measurements will be made individual settlement sites. Several simulation models will be used to estimate the local and regional effects on productivity and wildlife populations.

9901899 Molecular Mechanisms of Leaf Blade Development

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Strengthening Award; Grant 99-35304-8193; $100,000; 2 Years

Genes controlling the basic patterns of plant growth and development (shorter stature, earlier maturity, and bush vs. vine architecture) have been used extensively by breeders to tailor crop varieties for specific environmental niches. Research on cloning and molecular analysis of the genes involved will allow us to make these modifications more efficiently through genetic engineering of existing strains. This improves our response time to new agricultural problems and enhances the competitiveness of our domestic agricultural system. Our research is focused on genes controlling leaf development, using diploid *Nicotiana* as a model system. We recently isolated the *NTPHAN* gene from *Nicotiana tabacum* which is involved in leaf initiation and development of the leaf blade. Analysis of the DNA sequence shows a high degree of homology to the corresponding *PHAN* genes of maize, *Antirrhinum* and *Arabidopsis* indicating conservation of this function in a range of distantly related plants. We are investigating the function of this gene (1) by disrupting its expression with an antisense *NTPHAN* transgene, and (2) by extending its expression with a sense *NTPHAN* transgene. Results from antisense disruption indicate that *NTPHAN* is essential for proper development of photosynthetic cell layers near the upper surface of the leaf. Sense over expression of *NTPHAN* appears to produce a reiteration of photosynthetic cell layers on the under surface of the leaf. Our current work is aimed at elucidating its normal expression pattern in the leaf and investigation of *KNOX* genes as potential targets of *NTPHAN* regulation.

9901040 Late-season Fertilization Effects on Turfgrass Growth and Nitrate Leaching

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Strengthening Award; Grant 99-35102-8598; $85,000; 2 Years

A significant portion of the managed landscape in Connecticut and other Eastern Seaboard states is covered with turfgrass. To maintain turfgrass vigor and quality, fertilizer is applied routinely. Late-season or "fall" fertilization of cool-season turfgrass is a well-established management practice throughout much of the northern United States and is usually beneficial for turf quality in most cases. Although late-season fertilization provides turf quality benefits, an appropriate cut-off date for the last fertilizer application has not been identified for most regions. Considering that climatic conditions during the fall period vary considerably from region to region, it can be reasoned that the last date of fertilizer application to turf should also vary accordingly. This, however, is not the case and fertilizer recommendations for many areas typically call for a November application or are vague as when to apply the last application, often just using the term "fall". Applying fertilizers beyond the date when cool-season turfgrasses are becoming dormant increases the potential of nitrate losses in runoff and leaching. The main goals of this study are to determine an appropriate cut-off date for fall fertilization of turf grown in southern New England that maintains acceptable turf quality while minimizing nitrate leaching losses. Fertilizers need to be suitably applied to turf because inappropriate or untimely applications may contribute to the nutrient enrichment of receiving surface and ground water.

9901870 Molecular Interactions During Import of Thylakoid Proteins

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Strengthening Award; Grant 99-35304-8095; $120,000; 2 Years

The long range improvement and sustainable productivity of agriculturally important crop and forest plant species require a fundamental understanding of processes that are critical to the development and vitality of these species. This includes understanding the mechanisms involved in protein trafficking within plant cells. All proteins must be correctly targeted and localized in order to perform their functions at optimal levels. Thylakoid proteins, which serve in the conversion of light energy to chemical energy and in the process of evolving oxygen, are instrumental to the growth of the plant. Yet these proteins are correctly routed to the thylakoid against several odds: most are nuclear-encoded, must cross multiple intraorganellar compartments, and they must be specifically recognized by one of at least four different thylakoid transport pathways in operation. The goals of our studies are to elucidate how proteins are specifically recognized and transported by the Sec pathway and distinguished from proteins that utilize other thylakoid transport systems. CPSecA is a key component which may target proteins and initiate membrane translocation in the Sec pathway via recognition of their amino-terminal signal peptides. We will study the specificity of the interaction of CPSecA with signal peptides using *in vitro* biochemical assays and purified components. The molecular outcome of this interaction will be evaluated in terms of changes in the ATPase activity, stability, and conformation of CPSecA. Understanding how thylakoid proteins utilize the proper transport pathway to achieve their final destination successfully and efficiently is an important step toward elucidating mechanisms for enhancing vigorous plant life.

9901549 Investigating the Basis of Lipid Oxidation Induced Oxymyoglobin Oxidation

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Strengthening Award; Grant 99-35503-8132; $130,000; 2 Years

The ferrous, oxy-form of myoglobin (OxyMb) provides the desirable cherry-red color critical to consumer acceptance of fresh meat products. Oxidation of OxyMb to ferric brown metmyoglobin (MetMb) is associated with meat discoloration and results in lost value due to price reductions of whole muscle cuts, or grinding for rapid sale. It has been estimated that improved color stability in beef alone could result in $600 million added value to the meat industry annually. Oxidation of lipid in meat leads to rancid flavors and the production of several reactive products. There appears to be a relationship between discoloration and lipid oxidation in meat. We propose to test the hypothesis that a specific group of lipid oxidation products, , -unsaturated aldehydes, bind to OxyMb resulting in altered protein structure and enhanced MetMb formation. The proposed objectives will define the temporal relationship of aldehyde adduction with OxyMb oxidation, establish the specific amino acids that are adducted, and characterize physical alterations in protein structure caused by adduction. The implication is that lipid oxidation products may enhance discoloration in meat by altering the structure of this protein. Results will elucidate the specific effects of adduction on heme protein oxidation and provide a basis for better understanding lipid:protein oxidation interactions critical to functionality.

9903734 A Bench-Top GC/MS for Plant Biology Research

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Equipment Grant; Grant 99-35311-8407; $48,465; 1 Year

Gas-chromatograph/mass spectrometry (MS) is considered to be the most accurate and reliable method for separating, identifying and quantifying chemical compounds in higher plants and in bacterial pathogens. We are proposing the purchase of a GC/MS system in order to measure several distinct types of molecules, including various plant hormones, monosaccharide derivatives and acyl homoserine lactone pheromones. An independent mass spectrometry facility is highly desirable for our group of faculty at the University of Connecticut because of the large number of samples to be analyzed routinely, the low abundance of the compounds to be measured (requiring high instrument sensitivity), and the extensive amount of time required for methods development. Thus, a readily accessible GC/MS system, dedicated to our projects focused on molecular mechanisms of plant growth and development and agricultural crop improvement, should make our laboratories more productive.

9903659 Animal Nutrition and Antibiotic Investigations

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Equipment Grant; Grant 99-35208-8436; $26,647; 1 Year

The addition of a high performance liquid chromatograph (HPLC) with photo-diode array and fluorescence detection and autosampling is essential to the research goals of the Animal Nutrition group at the University of Connecticut. This instrument will permit immediate progress in the analyses of serum -tocopherol and plasma ascorbate concentrations in exercising horses, in the study of amino acid metabolism in dairy cattle, and for investigations concerned with antibiotic residues in milk from dairy cattle. Analysis of tocopherol and ascorbate concentrations in exercising horses is critical to understanding the effect of stress-associated oxidative challenge and antioxidant status. In dairy cattle, the effect of specific amino acids on the rate and extent of tissue mobilization to meet the energy needs of high producing dairy cows has not been adequately addressed, and understanding this effect will allow appropriate nutritional manipulation to maximize milk production. Finally, in related work, HPLC analyses of Beta Lactam antibiotics in milk is necessary for testing efficacy of a variety of rapid screening tests currently marketed to the producer. Validation of these tests will aid in assuring human food safety. The results of these projects will provide new information to the livestock industry, permitting appropriate strategies for the use of these supplements and pharmaceuticals.

9903624 Characterization of Cold Temperature-induced Proteins in *Yersinia enterocolitica* and the Effect of Osmolytes on its Cold tolerance

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Seed Grant; Grant 99-35207-8546; $49,412; 2 Years

Yersinia enterocolitica is a food-borne pathogen that has been implicated in disease outbreaks involving a variety of foods. One of the important characteristics of *Y. enterocolitica* is that it can grow at low temperatures. This is a major concern since refrigeration is the most common method employed for controlling growth of pathogenic microorganisms that cause spoilage in foods. Furthermore, consumer preferences for ready-to-eat meals, especially preservative-free chilled foods, have resulted in an expanded use of refrigeration for food preservation. This highlights the significance of *Y. enterocolitica* and the necessity for means to control it in refrigerated foods. In order to develop effective preservation strategies to prevent growth of microorganisms at low temperatures, it is critical to understand the mechanisms involved in their cold tolerance. Although the cold shock response has been studied extensively in *E. coli* and to a limited extent in food-borne pathogens such as *L. monocytogenes*, *B. subtilis*, and *V. vulnificus*, information on the physiological and genetic mechanisms involved in the cold tolerance of *Y. enterocolitica* is scarce. The objectives of this research proposal are to identify and characterize the protective proteins induced in *Y. enterocolitica* following exposure to a temperature downshift from 32° to 5°C and during continuous and balanced growth at 5°C in a defined minimal medium, milk and pork. The proposed research is expected to yield information on the survival and growth of *Y. enterocolitica* in refrigerated foods, thereby helping food scientists to devise more effective methods to control this pathogen.

9903522 Genetic Dynamics of the Weed Seed Bank: A Tool for Weed Management

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Seed Grant; Grant 99-35106-8246; $50,000; 2 Years

The widespread occurrence of herbicide resistance in weed populations has become a critical problem for agricultural weed management. This resistance represents genetic change in weed populations over years of herbicide use, since only those plants that are genetically resistant to herbicides can survive and reproduce. This project investigates an

aspect of weed biology that may provide a valuable tool for combating herbicide resistance: the seed bank.. Weed species produce numerous dormant seeds that are stored in the soil. These buried seeds from a reservoir of genetic variation drawn from earlier generations in the same site, including generations that predate herbicide treatment. Simply releasing such pre-resistance biotypes into the current breeding population through soil tillage may provide an effective, inexpensive, and environment friendly strategy to reduce or undo herbicide resistance in weed populations. To evaluate and develop this potential management strategy requires knowledge of (a) the amount of genetic variation stored in the weed seed bank, and (b) the extent that tillage effectively re-introduces this variation into breeding populations. We will answer these questions through direct genetic screening of a representative weed species, Chickweed. We propose a multidisciplinary "molecular ecology" approach that makes use of a fast, inexpensive DNA screening technique (Random Amplified Polymorphic DNA or RAPD) to determine the genetic variation and dynamics of the Chickweed seed bank in tilled and untilled plots of long-term field populations. The information produced will inform the design of alternative, sustainable weed management strategies.

9900795 Dietary Conjugated Linoleic Acid as a Novel Antithrombotic Agent

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Strengthening Award; Grant 99-35200-8580; $130,000; 2 Years

Numerous studies have been carried out to assess the role of dietary fat and fatty acids in the modulation of risk factors for cardiovascular and other diseases. Conjugated linoleic acid (CLA) has recently received considerable attention in view of its chemoprotective properties in animal models with various tumors or atherosclerotic lesions. CLA has been found predominantly in meats and dairy products and is a mixture of several positional and geometric isomers of linoleic acid (LA) containing conjugated double bonds. We have obtained *in vitro* evidence that CLA inhibits both human platelet aggregation and formation of the pro-aggregatory thromboxane A2 and that CLA is more potent than the corresponding nonconjugated LA. To further delineate this potential antithrombotic role for CLA, this proposal will determine whether or not CLA can alter the platelet anti-aggregatory potential of the endothelium. In view of the well-established protective role of prostacyclin in vascular tone and hemostasis, the *in vitro* study will focus on the effect of CLA on 1) endothelial (EC)-induced inhibition of platelet aggregation, 2) EC prostacyclin formation in EC-platelet mixtures and 3) EC prostacyclin formation via both the constitutive as well as inducible-prostanoid pathways. The effects of both CLA and LA will be compared to assess the importance of a conjugated double bond relative to a nonconjugated 1,4-diene functionality. If the proposed research yields additional evidence for an antithrombotic effect of CLA, this could provide justification for inclusion of dietary CLA as a fatty acid component in foods.

9903321 DNA Arrays to Monitor Host Gene Responses to Herpesvirus Infection

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Strengthening Award; Grant 99-35205-8228; $199,961; 3 Years

The objective of this grant is to better understand the avian herpesvirus, Marek’s disease virus (MDV). This virus causes both suppression of disease resistance and cancer in infected chickens. MDV is found in all large-scale chicken production facilities. Consequently, all commercially produced birds are immunized to prevent an outbreak of Marek’s disease. While the disease is currently controlled by immunization, strains of MDV are emerging which are resistant to current vaccines. Thus, a better understanding of how to control Marek’s disease is required. Our approach is to apply high-throughput gene screening to determine how the host responds to infection with MDV. By the use of DNA arrays containing thousands of genes, host gene expression that is either increased or decreased by MDV infection will be identified. The identity of the responding genes will be determined by rapid, automated DNA sequencing. The results may indicate how MDV suppresses disease resistance and induces tumors simultaneously. It may also provide insights to better control all herpesvirus infections.

9901519 High Performance Tangential Flow Filtration for Production of Purified Whey Proteins

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Strengthening Award; Grant 99-35503-8167; $170,000; 3 Years

Whey is a relatively inexpensive natural source of a wide range of valuable proteins with unique nutritional, functional, and therapeutic properties. However, these unique characteristics are largely unrealized in existing whey products due to adverse interactions between components in these complex mixtures. Several recent studies have demonstrated that High Performance Tangential Flow Filtration can potentially be used for the cost-effective purification of complex protein mixtures. The overall objective of this research is to examine the application of this emerging membrane technology for the purification of individual whey proteins. Protein separations will be achieved in this work by proper control of: (1) transmembrane pressure to achieve the optimal filtration rates, (2) solution pH and ionic strength to maximize

differences in the effective hydrodynamic volume of the individual charged proteins, (3) membrane charge to enhance the electrostatic exclusion of the more retained proteins, and (4) diafiltration conditions to achieve the optimal combination of yield and protein purification. Experiments will be performed with model systems consisting of mixtures of individual whey proteins and with actual whey protein concentrates. This research should provide a foundation for the development of new membrane systems for the purification of individual whey proteins with enhanced commercial value. In addition, this work should establish a framework for the application of High Performance Tangential Flow Filtration to the purification of other agriculture protein products (e.g., egg white proteins).

9901679 A Novel Integrated Process for Pilot-Scale Production of Xanthan Gum

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Strengthening Award; Grant 99-35503-8664; $140,000; 3 Years

Xanthan gum, the most important microbial polysaccharide, is widely used as a suspending and thickening agent in the food, chemical, and pharmaceutical industries. However, current industrial production using conventional stirred-tank fermentors is energy-intensive and costly. This is mainly because the high viscosity causes agitation and aeration to be difficult and limits the final yield and productivity. The problem encapsulates challenges in both fermentor design and operation, the key areas in fermentation process development. Our previous work has shown that, by using a novel centrifugal, packed-bed bioreactor (CPBR), xanthan productivity can be elevated significantly. Moreover, incorporation of membrane ultrafiltration (UF) to concentrate fermentation broth before alcohol precipitation can save up to 80% of energy used in downstream recovery. In this proposal, we consider not only the engineering challenges inherent in many scale-up operations but also the quality and functionality of the product produced from the new process, mainly the rheological characteristics of xanthan polymers. The overall project goals are to: (1) design and optimize the CPBR for pilot-scale xanthan gum production; (2) engineer the performance of UF process to further improve xanthan gum recovery; and (3) evaluate and control the quality and functionality of xanthan polymers produced from this process. Not only will this research project improve our knowledge on the scale-up operation of a novel integrated fermentation process for xanthan gum production, but it will also provide insights on critical issues associated with cell metabolism and product formation kinetics.

9900886 Reciprocal Weed/Crop Interaction as Influenced by Crop, Planting Date, and Weed Species

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Strengthening Award; Grant 99-35315-8145; $80,000; 2 Years

The yield reduction caused by a particular weed species depends not only on density, but also on other factors which affect crop and weed growth through time. Manipulating the time of crop planting date alter the impact of weed competition on crop yield, and in turn influence the intensity of weed management to eliminate yield loss. A majority of weed competition studies were conducted with corn or soybean. There has been no attempt to transfer our understanding of this body of yield loss data to less competitive and less acreage crops like vegetables and to determine if a relationship exists for a broader interpretation of existing databases. To bridge this knowledge gap, a study to understand the relationship between planting dates (mid May, mid June, or mid July), crops with different competitiveness (soybean, snap bean, and sweet corn), and weeds of variable growth characteristics (common cocklebur, jimsonweed, and velvetleaf) on reciprocal growth, development, and yield will be conducted at Georgetown, DE. Additionally, weed seed production and seed viability as influenced by planting dates and the appropriateness of transfer of yield loss data from soybean to less competitive snap bean and sweet corn will be evaluated. Information from this research on changes in weed/crop interaction and weed seed production as planting date changes can be fed into the economic threshold models to make cost-effective weed control decisions. Also, this research will derive a significant impact on weed management by decreasing inputs through an understanding of planting time effects on weeds.

9903709 Autogen 850 for Automated Plant DNA Extractions

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Equipment Grant; Grant 99-35311-8652; $50,000; 1 Year

The effective implementation of DNA markers for plant selection requires efficient means of DNA extraction from plant material. Indeed, the extraction of DNA is the bottle neck in the operation. We are part of a network of six research groups investigating the genetics of oil deposition in maize. One of our long term objectives is to develop new maize inbred varieties with elevated levels of oil that could be suitable for the production of high-oil hybrid seed. However, an least eleven genes contributing to the high-oil in phenotype segregate in progeny derived from a cross between Illinois High Oil (IHO) and Illinois Low Oil (ILO). The large number of genes involved in oil deposition in maize make the task of the breeder particularly difficult. To effectively reach our objective, it is expected that DNA markers will be needed to assist plant

selection efforts. The instrumentation we will purchase with this award will establish a DNA marker laboratory to support our plant breeding program and perform basic genetic linkage studies.

9903277 Production of All-Female Stocks of Pacific White Shrimp

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Strengthening Award; Grant 99-35206-8258; $130,103; 2 Years

The objective of this project is to establish methods to produce all-female populations of Pacific white shrimp (*Litopenaeus vannamei*) to increase productivity of U.S. shrimp farms. Female white shrimp are 10-20% larger than males at harvest. Our long-range goals are to develop economically viable methods of producing all-female shrimp for U.S. shrimp farmers. We propose to produce all-female shrimp populations by: 1) application of hormones in the water or feed; 2) manipulation of environmental conditions that may affect sex ratios; and 3)determination of family differences in sex ratios and selective breeding for skewed sex ratios. These approaches may allow for the commercial production of all-female shrimp populations as is typically done in salmonid farming.

The U.S. has an annual trade deficit in shrimp products in excess of $2.5 billion. With commercial shrimp fisheries at maximum sustainable yield, increased domestic production of shrimp will likely come from aquaculture. Currently, shrimp farmers rely on outdoor, earthen ponds situated along the coast. These farms are marginally profitable and there is little room for expansion of the industry. Future expansion of the U.S. shrimp farming industry may be dependent on biosecure production systems built away from the coast with little negative environmental impacts. Production of larger, all-female shrimp will increase the profitability of current shrimp farms, as well as the emerging inland, biosecure production systems. Increasing profits and productivity in these more environmentally friendly systems may allow for future expansion of the U.S. shrimp farming industry to all areas of the country

9900700 Japanese and White Female Adolescent Maturation

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Strengthening Award; Grant 99-35200-7619; $220,000; 2 Years

Prevention of osteoporosis requires building as much bone as possible during the adolescent period of rapid growth. Delaying the onset of menstruation (and the increased estrogen exposure associated with bone mass development) may reduce breast cancer. Prevention of these two chronic diseases may be in opposition, estrogen being “good” for bone but “bad” for breast cancer. Our objective is to identify modifiable factors in diet and physical activity that simultaneously result in high levels of bone mass development and delayed estrogen exposure associated with the onset of menstruation, and the timing of influence of those factors. Six hundred girls of White and Japanese ethnicity will be studied at Kaiser Permanente clinics in an observational study of factors influencing age of onset of menstruation and bone mass. Dietary (soy, calcium, fiber, fat, protein and energy), physical activity and body size factors influencing pubertal stage, age of onset of menstruation and bone mass will be identified and their dietary, activity and body size characteristics will be compared with those in the lowest level of bone mass and age at onset of menstruation. This study will contribute to the prevention of cancer and osteoporosis by identifying the relative contribution of dietary, physical activity, and body size factors to female adolescent maturation.

9901761 Structure and Function of Protein Disulfide Isomerase in Higher Plant Chloroplasts

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Strengthening Award; Grant 99-35306-8648; $100,000; 2 Years

This project focuses on the regulation of photosynthesis, the process by which plants produce oxygen and convert light energy into carbohydrate, protein and oils. Photosynthesis occurs on the photosynthetic apparatus located in subcellular structures, the chloroplasts. This apparatus is composed of the enzymes, factors and light-absorbing pigments needed for photosynthesis. The proper development of the photosynthetic apparatus is indispensable for crop productivity. High levels of sunlight and ultraviolet-B radiation (UV-B) reduce photosynthesis by damaging a protein, termed D1, in the core of the apparatus that is crucial for photosynthesis function. To maintain photosynthesis activity, plants have the ability to synthesize more D1 to replace the photo-damaged version. This "repair" process keeps the system operating. However, when the rate of photo-damage exceeds the rate of D1 synthesis, photosynthetic productivity is inhibited. This project uses molecular biological methods to determine the mechanisms that control D1 synthesis. We have recently isolated DNAs from genes for a key enzyme, protein disulfide isomerase (PDI), which is believed to regulate the rate of D1 synthesis - acting like a throttle - in response to light. This project will isolate the entire gene for PDI and the genes for other proteins needed for PDI function, and determining how these proteins function in the *Arabidopsis* plant. *Arabidopsis* is the most well-developed plant genetic system available. Research on this plant has yielded information applicable to crop plants. Isolating the PDI genes is a prerequisite which may allow us to manipulate photosynthetic productivity via biotechnology.

9900897 Temperature and Light Regulation of Purple Nutsedge Sprouting and Management

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Strengthening Award; Grant 99-35315-8146; $100,000; 2 Years

Scientists who assessed the world's major weeds have designated purple nutsedge as the world's worst weed. Dormancy and erratic emergence are key actors in purple nutsedge survival and invasiveness in crops. However, the lack of understanding of these processes makes purple nutsedge management extremely difficult. Apical dominance of tubers in chains (chain dormancy) and diurnally Fluctuating temperatures are probably the most important: factors controlling dormancy and sprouting of purple nutsedge tubers. Therefore, the overall goal of this project is to provide a working knowledge of how the key environmental factors control purple nutsedge tuber sprouting in chains and as individual tubers.

Our previous research showed that breaking chain dormancy and exposure of tubers to diurnally fluctuating temperature can stimulate all tubers to produce sprouts within 3 weeks. Temperature variations regulate both tuber budbreak and bud elongation. However these studies were conducted with one purple nutsedge clone. In order to expand the relevance of our findings, the proposed research will determine whether the fluctuating temperature control of tuber sprouting is a common phenomenon to purple nutsedge ecotypes from diverse geographical sites. We also expect to establish that chain dormancy is mediated by different levels of radiant energy, and that crop canopy can increase chain dormancy and suppress tuber sprouting. An increased understanding of these processes will aid in the development of management strategies to control purple nutsedge and boost crop yields in a sustainable manner.

9903711 Upgrade of HPLC System for Carotenoid Analyses

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Equipment Grant; Grant 99-35106-8322; $18,957; 1 Year

Carotenoids are naturally-occurring lipid-soluble pigments that give flowers, tubers and roots of plants their yellow to orange color. Leaves also contain carotenoids but the yellow is masked by the intense green of chlorophyll. There are literally hundreds of carotenoids produced by plants and microorganisms and much is still unknown about their biological functions. Animals obtain carotenoids from their diet, the most important being p-carotene, the precursor of vitamin A. In leaves, carotenoids appear to have at least two functions, one as part of the light absorbing system for photosynthesis and the other as protective pigments against potentially damaging oxidation and excess light. The latter functions are crucial and explain the presence of carotenoids in all leaves. This laboratory is engaged in research on the xanthophyll cycle that operates in higher plants and algae to protect the photosynthetic apparatus against the damaging effects of high light by dissipating the excess energy as heat. The xanthophyll cycle is the only dynamic carotenoid cycle known. The objectives of our research are to understand the biochemistry and molecular biology of the xanthophyll cycle and to test the effects of modifying the cycle through genetic engineering. These studies require a high performance liquid chromatograph (HPLC) for the rapid and accurate analysis of the carotenoids. This grant will be used to upgrade an existing HPLC by replacing the 1 5-year-old detector for increased sensitivity and by adding an autosampler for improved efficiency.

9903563 Identification of Regulatory Elements in Hormone-Regulated Larval Specific Genes from Tobacco Hornworm, *Manduca sexta.*

Lan, Q.
University of Northern lowa; Department of Biology; Cedar Falls, IA 50614-0421

Seed Grant; Grant 99-35311-8313; $45,000; 2 Years

This research proposal aims at a deeper understanding of developmental-specific gene regulation by insect hormones. Insect's growth and metamorphosis are under the regulation of two major hormones, ecdysteroid and juvenile hormone (JH). Two of the developmental-specific genes have been isolated from *Manduca sexta,* the insecticyanin A (INS-A) and LCP 14 genes. Ecdysteroid indirectly switches on and off these genes during larval molts and metamorphosis, and juvenile hormone determines whether ecdysteroid induced gene expression changes are transient or permanent. However, the regulatory mechanisms whereby this occurs are unknown. The goal of the experiments proposed here is to identify the relevant regulatory sequences in these genes and the hormone-induced nuclear factor(s) that interact with those sequences. The regulatory DNA sequences in both genes will be linked to a reporter gene, and the expression of the reporter gene under the control of INS-A or LCP14 will be examined in cultured *Manduca sexta* cells using DNA transfection technique. Nuclear proteins that interact with those DNA regulatory sequences will be identified in nuclear protein extracts from specific development stages of *Manduca sexta* using gel mobility shift assays.

My long-term research goal is to reveal the mechanisms that regulate insect growth and development by insect hormones at the molecular and cellular levels. This study may provide new information on JH-directed cellular development and coordination of hormones in regulating gene expression. Understanding the molecular mechanisms underlying the action of insect growth regulators, especially the insect hormones, is essential for the development of new insecticides.

9903662 High-Performance Liquid Chromatography
Schimpf, M.E.; Ellis, R.W.; Bammel, B.; Carter, L.
Boise State University; Department of Chemistry; Boise, ID 83725
Equipment Grant; Grant 99-35208-8583; $38,397; 1 Year

The primary expense in raising trout is the cost of feed. The most critical and expensive component of the feed is protein, which supplies the essential amino acids for growth. A method for continually monitoring amino acid levels in the blood and tissue of live trout and salmon will be developed. Since temperature differs among trout hatcheries and changes during the downstream migration of salmon through reservoirs, the method will be used to evaluate changes in the protein requirements of trout and salmon with temperature. A metabolic chamber for holding the fish will be built, and a tiny probe will be used to sample blood and tissue-fluid in live fish. The probe will be interfaced to the liquid chromatograph, which will quantify specific amino acid levels. With this integrated device, the specific uptake and consumption of proteins by liver and muscle will be monitored with changes in diet and temperature. The objective is to identify optimal diets in trout, and the effect of temperature on such diets, and to identify pre-release diets that increase the survival rate of salmon during their downstream migration through reservoirs.

In a separate project, the liquid chromatograph will be used to isolate and quantify a toxin produced by cheat grass to eliminate competing grasses. Cheat grass has low nutritional value and a tendency to fuel range fires. This work is of fundamental importance in developing strategies for the elimination of cheat grass and the recovery of Idaho's native grasses.

9901010 Closed-Loop Precision Irrigation for Improved Water Management
King, B.A.; Wall, R.W.; Stark, J.C.
University of Idaho; Idaho Agricultural Experiment Station; Moscow, ID 83844-2337
Strengthening Award; Grant 99-35102-8575; $98,000; 2 Years

One of the technological barriers to commercialization of precision irrigation is the lack of a cost effective means for determining spatially variable irrigation requirements. This project targets the development, testing, and evaluation of a closed-loop decision support system to effectively determine spatially variable irrigation requirements. The system consists of spatially distributed micro-instrumentation units linked to a center pivot supervisory control and data acquisition network using a low-power radio frequency link. The micro-instrumentation units will be equipped with sensors to monitor soil water content, water application, and environmental parameters within and above the crop canopy. Data collected by the micro-instrumentation units will be maintained at the center pivot control point for retrieval by a portable computer. A simple decision support model for determining spatially variable irrigation requirements will be developed. The micro-instrumentation units linked to the center pivot control and data acquisition network will provide near real-time information to drive the decision support system. The closed-loop control system will be developed and tested using data from artificially induced spatially variable irrigation requirements under controlled field conditions. Performance of the control system will be evaluated based on ability to maintain target soil moisture levels under induced variability and resulting crop yield and quality.

9902444 Benzenediols and Benzoquinones: Roles in Wood Decay by Brown-Rot Fungi
Crawford, R.; Paszczynski, A.
University of Idaho; Environmental Biotechnology Institute; Moscow, ID 83844-1052
Strengthening Award; Grant 99-35103-7997; $150,000; 2 Years.

Lignocellulose is among the most abundant, renewable natural materials on earth. As wood, lignocellulose provides a versatile building material, an important fuel for many societies, and an important reservoir of carbon for the earth's carbon cycle. The lignin in plant tissue is an important source of stable organic matter in soils, especially undisturbed forest soils, but also most agricultural and grassland soils. Biological decay of wood causes losses of billions of dollars to the United States. Thus, an understanding of the processes involved in biodegradation of lignocellulose is important across many disciplines of science and engineering. One of the major forms of wood decay is fungal brown rot. The specific mechanisms of brown rot are not well understood. The research performed during this project will lead to an improved understanding of biochemical mechanisms of brown rot decay, and should lead to strategies for its control. We will determine whether or not certain benzenediols and benzoquinones produced *de novo* by the brown rot fungus *Gloeophyllum trabeum* are involved in lignocellulose catabolism through promotion of oxidative Fenton's reactions. We will examine approaches to inhibit these reactions and determine if these approaches can be used to prevent or decrease brown rot decay.

9903591 Acquisition of a Super-Critical CO_2 Lipid Extractor
Hardy, R.W.
University of Idaho; Hagerman Fish Culture Experiment Station; Hagerman, ID 83332
Equipment Grant; Grant 99-35208-8549; $15,730; 1 Year

This equipment grant request was made to acquire a super-critical CO_2 lipid extractor for use in research studies in fish nutrition and fish feeds associated with aquaculture. Lipid extraction and quantification is a component of proximate

analysis of foods, feeds, feed ingredients, and fish tissues, and is widely used in animal and fish nutrition research. The super-critical CO_2 lipid extraction apparatus can determine the lipid (fat) content of 60 samples per day compared to six samples per day with existing equipment at the same price and accuracy per sample. Lipid is removed from samples with CO_2 gas under high pressure where it becomes a liquid, instead of by heated organic solvents in conventional lipid extraction. This eliminates safety, storage, and disposal concerns associated with the use of organic solvents. The new equipment will be used in existing and future research studies aimed at improving the efficiency of fish farming and at reducing the impact of fish farming on the aquatic environment through the development of environmentally-friendly fish feeds.

9902523 The Spectra of Variants in Potyvirus Populations

Berger, P.H.
University of Idaho; Department of Plant, Soil and Entomological Sciences; Moscow, ID 83844-2339
Strengthening Award; Grant 99-35303-8632; $100,000; 2 Years

The existence of RNA viruses as quasispecies - a mixture of viral sequences containing a predominant sequence, and a variable mixture of related but molecularly distinct sequences - has observed for a number of mammalian and bacterial viruses. These viruses appear to evolve at a very rapid rate because of the lack of proofreading of RNA-dependent RNA polymerases. Thus, these important disease agents can mutate at rates that are on the order of size magnitudes greater than DNA-based organisms. However, little data are available for the plant viruses. This proposal is aimed at obtaining information on sequence diversity in certain populations of legume-infecting potyviruses, and determining the nucleotide substitution frequencies in these populations. The extent and nature of nucleotide sequence variants in populations of certain legume-infecting potyviruses will be examined. As these populations are characterized, we hope to then determine how and at what rate population structure may shift, over time, in response to selective pressures, such as host plant resistance. Understanding the nature of potyvirus evolution will provide insights as to how these viruses may respond to various environmental and selective factors in nature. Ultimately, we distributions of molecular sequences and its fitness. Since these are among the most economically important viruses, this information may facilitate classical and biotechnological breeding for virus resistence in crops.

9902318 Immunosuppression by Host-Specific Staphylococcal Enterotoxin C in Mastitis

Bohach, G.A.
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Strengthening Award; Grant 99-35204-8581; $180,000; 2 Years

Staphylococcus aureus is a bacterium that causes important diseases in humans and animals. Bovine mastitis is one infectious disease caused by this bacterium with a major economic impact for producers. Despite many attempts to control staphylococci in dairy herds, mastitis remains a major problem. Our hypothesis is that toxins produced by staphylococci in the mammary gland suppress the bovine immune response and prevent elimination of the organism. We have shown that one common toxin, staphylococcal enterotoxin C (SEC), produced by many mastitis strains, causes the bovine immune system T cells to function in a way that could cause immunosuppression. The goals of this present study are two-fold. First, experiments will investigate the ways in which SEC causes a relative decrease in helper T cells. This work will identify cellular targets of the toxins and provide information that could lead to methods for protecting the animal. The second objective is to determine the types of toxins involved in bovine mastitis. This objective is important since staphylococci are known to produce at least 10 different types of toxins with properties similar to SEC and several new toxins have been discovered in recent years. We will survey at least 500 staphylococcal isolates from various geographical areas around the world for toxin production. Combined, these studies will define the role for certain types of bacterial toxins in bovine mastitis. If this study implicates immunosuppressive toxins in mastitis, new targets for treatment and prevention will be made available to the dairy industry.

9903660 Acquisition of a Rapid Visco Analyzer for Characterization of Starch Properties

Huber, K.C.
University of Idaho, Moscow; Department of Food Science and Toxicology; Moscow, ID 83844-1053
Equipment Grant; Grant 99-35501-8662; $25,000; 1 Year

Starch rheological behavior is an important discriminating property that ultimately dictates the relative value and end-use potential of cereal commodities, flours, and isolated starches. The Rapid Visco Analyzer (RVA) has recently emerged as a standard instrument for evaluating the gelatinization and subsequent pasting behavior of native and modified starches, and has become an indispensable instrument for cereal and starch chemists alike.

The acquisition of a RVA will dramatically broaden laboratory research capabilities and provide a means for researching topics of critical importance. Central research topics, which necessitate a RVA instrument, include (1) investigation of starch chemical modification, (2) identification of factors relating to cereal and starch quality, and (3) evaluation of emerging cereal cultivars (arising from plant breeding programs) for end-use potential and value. For each

research category, the RVA will play a central role in relating intrinsic flour/starch characteristics to observed behavioral properties to promote greater understanding of structure/function relationships and issues of cereal quality. Proposed projects are designed to increase function, quality, understanding, and utilization of cereals and cereal products. As a new investigator, the RVA instrument will not only provide indispensable rheological support to current projects, but will also facilitate the expansion, competitiveness, and long-term stability of a developing research program by broadening the scope of laboratory research capabilities, increasing the potential for multidisciplinary collaboration, and enhancing the ability to generate preliminary data necessary to procure research funding.

9903665 Atomic Absorption Spectrophotometer for the Center of Excellence at Southern Illinois University, Carbondale

Apgar, G.A.; Griswold, K.E.; Varsa, E.C.
Southern Illinois University; Department of Animal Science, Food and Nutrition; Carbondale, IL 62901-4417
Equipment Grant; Grant 99-35208-8536; $25,050; 1 Year

Quantification and realization of nutrient flow in the agriculture sector is of growing interest. With the acquisition of a new atomic absorption spectrophotometer, our Center of Excellence for Soybean Research, Teaching and Outreach will be able to investigate numerous facets of nutrient conservancy from our livestock and crop production units. Currently we are researching the role of the enzyme phytase to liberate phosphorus and trace minerals from swine diets containing wheat middlings. Wheat middlings are a dietary component that contains a high concentration of phosphorus, although not all of it is available to the pig for utilization. By enabling the phosphorus found in the dietary components to be absorbed, it significantly reduces the concentration of nutrients in the effluent. Along these same lines, we are studying the ability of phytase addition to allow the removal of phosphorus supplementation during the finishing period of swine production in an effort to reduce phosphorus output. Our research efforts are also directed toward the use of zebra mussels for bio-filtration of swine effluent, and the study of soil nutrient concentrations and their affects upon nutrient availability to plants. Hence, nutritional alterations, innovative effluent treatments and applications with respect to important nutrients will enable our university to track nutrients, and model nutrient flow. This information may aid the modeling process to account for nutrient flows in both crop and livestock production areas. The Center of Excellence has various research aims, and is founded by interdisciplinary and interdepartmental research faculty.

9903674 High Performance Liquid Chromatography for the SIUC Soybean Center of Excellence

Winters, T.A.; Banz, W.J.
Southern Illinois University; Department of Animal Science, Food and Nutrition; Carbondale, IL 62901-4417
Equipment Grant; Grant 99-35501-8663; $34,715; 1 Year

High performance liquid chromatography (HPLC) is an analytical method for measuring organic macromolecules in plant and animal tissues. We have developed a research program on the beneficial utilization of soybeans as a food and feed component which requires the availability of this technology. The purpose of this proposal is to obtain matching funds to purchase an HPLC to support research programs at Southern Illinois University Carbondale (SIUC), which have the primary objective of improving productivity and utilization of soybeans. A Center of Excellence for Soybean Research, Teaching, and Outreach at SIUC. One of the primary goals of the Soybean Center of Excellence is to enhance the research capabilities of SIUC by improving equipment and infrastructure. In this plan, the Center will develop core facilities that soybean researchers will utilize. HPLC has been determined to be a priority need by researchers in the Soybean Center. It will be utilized to analyze components of soybean and soy food and/or feed products, such as isoflavones (phytoestrogens), amino acids, phytosterols, vitamins, oligosaccharides, oligopeptides, and cytokinins. It will enable analysis of metabolites of these soybean components in the tissues (e.g. blood, urine, etc.) of animals and humans that consume soybean products. In addition to being utilized by SIUC soy researchers, the facility will provide analysis service on a contract basis to researchers at other universities and in industry. This HPLC system will give researchers at SIUC a competitive advantage in performing soybean research and obtaining additional financial support for this research.

9903643 Purchase of a Medium Pressure Liquid Chromatography System

Huang, J.K.; Wen, L.
Western Illinois University; Department of Chemistry; Macomb, IL 61455

Equipment Grant; Grant 99-35501-8312; $24,855; 1 Year

This equipment grant will purchase a medium pressure liquid chromatography system for proteins purification. Two projects in our laboratory will greatly benefit from the availability of the new equipment. The first project is the isolation and characterization of secondary alcohol dehydrogenase from *Nocardia cholesterolicum (NC)* NRRL 5767. This microbe is renowned for converting oleic and linoleic acids in corn and soybean soapstock to hydroxy fatty acids. However, the microbe also contains secondary alcohol dehydrogenase, which further converts hydroxyl fatty acids to corresponding keto fatty acids. The presence of keto fatty acids not only decreases the yields of more useful and valuable hydroxyl fatty acids but also increases the difficulty in purifying hydroxyl fatty acids. Isolation and characterization of secondary alcohol dehydrogenase are the first steps in our long-term project of genetically engineering NC NRRL 5767 lacking secondary alcohol dehydrogenase. These recombinant microbes will enable us massively to produce value-added hydroxyl fatty acids free of keto fatty acids in soapstock of corn and soybean.

Isolation of deoxyhypusine hydroxylase from bovine testes is the second project. Deoxyhypusine hydroxylase is essential for hypusine synthesis in eucaryotic initiation factor 5A. Inhibition of this enzyme has been shown to exert antiproliferative and antiretrovial effects in eucaryotic systems, and is regarded as potential targets for antiproliferative and antiretrovial therapy. Knowledge from structure-functional studies will contribute to achieving more specific inhibitors, which may better control hyperproliferative diseases in livestock.

9901451 Structure-Property Relationships in Polyurethanes Based on Vegetable Oils

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Pittsburgh State University; Kansas Polymer Research Center; Pittsburgh, KS 66762-7560

Strengthening Award; Grant 99-35504-7873; $165,000; 3 Years

Agricultural products are a source of many valuable chemicals. Vegetable oils have a number of excellent properties, which could be utilized in producing valuable polymeric materials. They can be transformed into polyols for general polyurethane use. Polyurethanes are a group of polymeric materials with many applications. Their current consumption exceeds 2.4 million tons per year in the United States. Replacing petrochemical raw materials with renewable resources such as vegetable oils is of significant economic, environmental and strategic importance, particularly in United States -- the world's largest producer of vegetable oils. One of the basic problems in using raw materials of vegetable origin, such as oils, is that their structure varies more than that of synthetic chemicals. This is reflected in the variability of structure and properties the polymer products based on vegetable oils.

The objective of the project is to study the effect of polyurethane network structure on properties of two groups of materials: rigid foams and cast resins. The network structure will be varied by varying the type of oils, the type and content of hydroxyl groups in the polyols, and the type of isocyanates. The oils to be used will include commercial vegetable oils such as soybean, corn, canola, rapeseed, olive, linseed, safflower, sunflower, peanut, cottonseed, and palm oils, as well as model triglycerides. The effect of polyurethane structure on physical, mechanical and electrical properties will be studied. The results of the study will help us control the properties of the oil-based plastic materials in the optimal way.

9901795 Characterization of Plasma Membrane F-actin Binding Proteins

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Strengthening Award; Grant 99-35304-8009; $138,000; 2 Years

The actin cytoskeleton is a spatially and temporally dynamic protein filament-based system which functions in a number of essential processes in all eukaryotic cells. In plant cells actin cytoskeletal dynamics are involved in a number of cellular processes which are critical for normal plant growth, development and thereby productivity. To meet the multiple demands for the actin cytoskeleton during the life of the cell, actin filaments participate in an elaborate choreography which involves their assembly, disassembly and association with other proteins to form arrays specialized for particular functions. Actin-binding proteins play an important role in regulating this dynamic behavior of the actin cytoskeleton. The goal of this research is to purify novel plant plasma membrane F-actin-binding proteins and then develop the molecular tools required to study their cellular functions. The specific objectives are to 1) purify plasma membrane-associated F-actin-binding proteins which have been identified using a F-actin gel overlay assay, 2) obtain the corresponding clones, 3) produce antibodies to these proteins and, 4) determine the cellular and subcellular location of these proteins. Completion of these experiments will contribute to our understanding of fundamental cellular processes which are essential for optimal plant growth, development and reproduction.

9901588 Designing Effective Environmental Labels for Forest Products

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Strengthening Award; Grant 99-35400-7851; $140,000; 2 Years

From a policy perspective, the eco-labeling of forest products can educate consumers about the environmental impacts of a forest products manufacture, leading to changes in purchasing decisions, and ultimately, to reductions in negative environmental impacts. From a business perspective, eco-labeling allows firms to be awarded for their environmentally responsible forest management practices. The wide spread use of eco-labels suggests that they are perceived as effectively altering consumer behavior. However, few studies identify the effectiveness of alternative eco-labeling programs and no research has been conducted on the optimal form of forest product labeling. The objectives in this research is to: 1) identify the types of environmental information that consumers will find useful when choosing among forest products; 2) understand how different ways of presenting the information affects consumers' ability to comprehend and utilize the information; 3) understand how different ways of presenting the information affects consumers' perception of products' environmental impacts; 4) understand how 1)-3) differ across different types of consumers. Through the use of various statistical techniques we will be able to identify not only the magnitude of each respondent's errors and the effect of format on calculation time and effort, but also to determine if these errors caused a choice inconsistent with personal preferences. Together these will provide an opportunity to estimate the policy effectiveness of various labeling policies and the welfare implications of different labeling strategies.

9903623 An Organophosphate Pesticide Sensor

Frankel, D.J.; Bushway, R.J.; Segee, B.
University of Maine; Laboratory for Surface Science & Technology; Orono, ME 04469-5764

Seed Grant; Grant 99-35501-8661; $49,704; 2 Years

With passage of the Food Quality Protection Act of 1996 (FQPA) pesticide tolerances are changing and the ability to rapidly detect organophosphate pesticides in our food supply is becoming increasingly important. An efficient way of screening food imported from countries lacking stringent pesticide laws as well as food produced in the United States is needed. With the development of national organic food standards and with the premium prices obtainable for organic products there is also a need to test organically labeled food for pesticides. Present testing methods, which use the enzyme cholinesterase in a reaction vessel or coated on a biosensor, are not ideal for rapid screening of food samples. We propose to develop a sensor array to rapidly detect organophosphate pesticides using Maine wild blueberries as a model system to demonstrate the feasibility of the technique. The sensor array will consist of surface acoustic wave devices (SAW's) coated with polymer materials chosen to selectively adsorb organophosphate. The individual sensors in the array will be coated with slightly different polymers since no one polymer material will adsorb only the desired pesticide. Upon adsorption of a small amount of vapor the SAW devices exhibit a change in oscillation frequency which is easily measured. Advanced signal processing of the sensor array output will be used to determine the presence or absence of two organophosphate pesticides azinphosmethyl and phosmet commonly used on Maine blueberries. A gas-phase sensor as proposed here has the potential to be faster and more easily miniaturized than existing technologies.

9903379 Improved Information in Support of a National Strategy for Open Land Policies

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University of Maine; Orono ME 04469-5782

Strengthening Award; Grant 99-35403-8658; $155,072; 2 Years

Nearly 200 state and local initiatives to curb sprawling development were on a November 1998 ballot (Washington Post). The Federal Agricultural Improvement and Reform Act of 1996 (FAIR) authorized up to $35 million (from Commodity Credit Corporation funds) for state and local farmland protection programs. More recently, Vice President Gore announced a $9.5 billion bond proposal to curb urban sprawl and proposed $1 billion in tax credits and grants to help communities preserve farmland (Gore). Given this broad perspective of open land protection, it is striking that the policy debates are missing reliable and consistent information about what, exactly, supporters of such programs really care about and value. This study is designed to identify and quantify public preferences for the provision of open land through agricultural lands, and will investigate how the quality of open land is affected by the agricultural system employed. The design provides policy relevant information regarding what attributes open land provided by agricultural lands the public prefers, the effects of the agricultural systems on the quality of open land and regional differences in public preferences.

The research proposed here will use qualitative research methods. The qualitative research will involve focus groups held in five locations throughout the U.S. The quantitative component will involve a mail survey that includes conjoint questions designed to learn what attributes of agricultural lands are important in providing open land and the economic value the public places on open land provided by agricultural lands. The integration of the results from the focus groups and mail surveys with a review of existing open land protection efforts will enable us to make design recommendations for the future programs, policies and legislation to protect open land through agricultural lands. This

integration will enable us to design policies and legislation so that public preferences will be addressed and the proposals will gain greater public support. This policy analysis will also make recommendations regarding what level of government should be involved in protection efforts, whether different levels of government should be involved in different issues relating to protection of agricultural lands for open land, and whether efforts should involve joint public, nonprofit, and private efforts. Finally, we will address how policies that effect agricultural systems can be developed to enhance the role of agricultural lands in the provision of open land.

9903650 Headspace Analyzer and Gas Chromatograph for Foods and Agricultural Products
Camire, M.; Skonberg, D.
University of Maine; Department of Food Science and Human Nutrition; Orono, ME 04469-5736
Equipment Grant; Grant 99-35501-8255; $27,029; 1 Year

Volatile chemicals released from foods and other products contribute significantly to consumer acceptance. Off-flavors produced from chemical or microbial changes can repel potential consumers. This proposal requests funds to purchase a Hewlett Packard gas chromatograph, a Tekmar-Dohrmann headspace analyzer and necessary support equipment such as detectors and computer interface. This equipment measures the volatile chemicals released from foods, and while the equipment cannot characterize these compounds as good- or bad-smelling, the information provided enhances the evaluations made by human volunteers. This equipment will be used several faculty working with a variety of foods. One use for this equipment is to study the effectiveness of antioxidants. Antioxidants exist naturally in or are added to foods and animal feed to prevent lipid oxidation, also known as rancidity. Oxidation is also involved with aging and many diseases such as atherosclerosis and cancer. The headspace analyzer will not only allow us to see how well antioxidants protect foods from rancidity, but also how consuming antioxidants protects people from oxidation damage. The equipment will also be used to detect off-flavors in foods coated with an edible film derived from crab shells; another project will develop more attractive feeds and bait for lobsters and other economically-important aquatic species. The state of Maine ranks 49th in research funding; this grant will assist food science and nutrition faculty in securing additional funding from federal, state and private sources.

9901830 Expansin Expression During Adventitious Rooting in Loblolly Pine
Hutchison, K.W.; Greenwood, M.S.
University of Maine; Department of Biochemistry, Microbiology and Molecular Biology; Orono, ME 04469-5735
Strengthening Award; Grant 99-35304-8115; $120,000; 2 Years

One of the most significant changes that occurs as conifers age is the loss of the ability of cuttings to form roots. In loblolly pine seedlings, a gradient in root forming ability is evident early in seedling development. While the primary root will readily form lateral roots, cuttings in the base of the stem (hypocotyl) will only form roots in response to the application of the plant hormone auxin. Cuttings in the upper part of the stem (epicotyl) form few, if any roots, regardless of whether auxin has been applied or not. We have determined that a gene family called expansin was expressed in loblolly pine hypocotyl cuttings during the first 12 to 24h after the application of auxin. Expansins are responsible for the loosening of primary cell walls, a necessary step for changes in development. We have also determined that expansin is expressed in cells from which roots will be formed. These data suggest that expansin is an essential early event in the formation of roots. To continue this work we plan to determine the genetic structure and the expression patterns of the individual members of the expansin gene family in loblolly pine from both rooting and non-rooting tissue. In addition, in other plants the direct application of expansin protein has been shown to effect development. We will also test this ability of expansin by direct application of the expansin protein to pine seedlings. Our expectation is that it will induce at least some of the necessary changes for root formation.

9900756 Impacts of CO and H_2 Production by Legumes on Soil-Atmosphere Trace Gas Exchange
King, G.M.
University of Maine; Darling Marine Center; Walpole, ME 04573
Strengthening Award; Grant 99-35107-8552; $90,000; 2 Years

The nodules of nitrogen-fixing plants (legumes) produce hydrogen gas, which is emitted to the atmosphere. Nodules are also capable of producing and emitting carbon monoxide (CO). CO is an especially important trace gas that plays a major role in atmospheric chemistry. Due to their broad distribution, their importance in agriculture and potential rates of CO production, legumes may have a significant impact on exchanges of hydrogen and CO between soils and the atmosphere. In addition, emission of both hydrogen and CO by nodules can affect rhizosphere microbiology by enhancing populations of trace-gas-consuming bacteria.

Since the mechanisms for CO production by nodules are unknown, we are undertaking an effort to determine the sources of nodule CO and controls of emission. Our research will investigate the potential for CO production by legume bacterial symbionts (e.g., *Rhizobium* spp.) *via* a side reaction of the enzyme, nitrogenase, or *via* degradation of the oxygen-carrying protein, leghaemoglobin. These assays will involve pure cultures of bacteria incubated under conditions that

promote nitrogen fixation, and in media with and without sources of haem. Assays will also include bacterial fractions isolated from active nodules. In addition, we will determine the extent to which leghaemoglobin recycling by plant cells contributes to CO production. These assays will be based on fractionation of nodules to produce bacteria-free preparations to which we will add various haem-containing proteins. Collectively, results from these studies and additional comparisons of activity under *in situ* conditions will establish the mechanisms by which legume nodules produce and emit CO.

9903621 An Impedance Based Ethylene Sensor for Determining Fruit Quality and Storage Life

Vetelino, J.F.; Bushway, A.A.
University of Maine; Laboratory for Surface Science and Technology; Orono, ME 04469

Seed Grant; Grant 99-35501-8659; $49,818; 2 Years

In the development of fruit, ethylene is known to be a direct measure of the ripeness. At the onset of the ripening process, the fruit emits concentrations of ethylene in the parts per billion range which increases to a maximum value in the parts per million range when the fruit is ripe. A low cost, deployable ethylene sensor would provide food producers, processors and retailers with an instrument which would allow fruits to be harvested, processed and/or retailed at their peak quality, thereby minimizing losses due to spoilage. At present, an ethylene sensor which can be employed in the field or in a mobile storage facility does not exist.

The main objective of the present proposal is to evaluate the feasibility of an impedance based sensor for the determination of fruit quality and storage life. This sensor represents a significant improvement over current sensor technologies in that it provides the user with three critical sensing parameters, namely, AC excitation frequency, and the real and imaginary parts of the impedance. This is in contrast to existing sensor technologies which provide only one critical sensing parameter. The specific objectives include identifying the sensor operating temperature, determining the sensitivity and selectivity of the film to ethylene, and applying the sensor to monitor the ripening process in bananas and tomatoes. It is anticipated that the proposed project will result in close collaboration between sensor and food scientists providing the basis for this interdisciplinary team to compete successfully for significant future funding relating to the sensing needs of the food industry.

9902423 Foaming of Wood-Fiber/Thermoplastic Composites

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New Investigator/Strengthening Award; Grant 99-35103-8601; $90,000; 2 Years

Wood-fiber/plastic composites make use of cellulosic fibers as fillers and/or reinforcing agents in the plastic matrix. These composites have a lower material cost and a higher stiffness than neat plastics. Although these composites have been commercialized, their potential for use in many industrial applications has been limited because of their brittleness, lower impact resistance, and higher density compared to neat plastics. The potential range of uses for these materials in innovative applications would be expanded if these shortcomings could be overcome. Recently, the concept of creating microcellular foamed structures in the composites as a means to resolve these shortcomings has successfully been demonstrated by the PI. Microcells have been developed in the composites through a batch foaming process by using carbon dioxide as a foaming agent. Because of the generated microvoids, the impact strength, brittleness, and density of the composites were dramatically improved. These enhancements were achieved without significantly compromising other mechanical properties. Despite these promising results, the batch foaming process used to generate cellular foamed structures in the composites cannot now be implemented in the industrial production of foams because it is not cost-effective. A procedure for continuous manufacture of foamed wood-fiber/plastic composites is required for the expanding commercialization of these products and is the focus of this study. The objective of the proposed investigation is to develop the scientific and engineering basis needed to manufacture wood-fiber/plastic composite foams using a continuous extrusion process. Positive results from this study are expected to lead to a new class of materials with unique properties and characteristics. Improving the shortcomings of wood-fiber/plastic composites will extend their utilization in a large number of innovative applications and increase the value-added utilization of wood.

9902652 Effect of Novel Fungal Endophytes on Pest and Beneficial Insects

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Strengthening Award; Grant 99-35302-8164; $100,000; 2 Years

Fungal endophytes of forage grasses have detrimental effects on livestock. Ergot alkaloids produced by the fungi appear to be the primary cause for these effects. In addition, fungus-produced loline and peramine alkaloids provide infected plants with enhanced resistance to insect herbivores. The fungi, and the toxins they produce, result in annual revenue losses of over $600 million in the USA current strategy by plant breeders is to develop grasses with fungal strains that reduce the negative effects on livestock but retain resistance to insects. The proposed research utilizes newly developed

experimental endophyte strains that were artificially introduced (i.e., novel) into two tall fescue cultivars and tests their effects on pest and beneficial insects. I will test for resistance to caterpillars and aphids in two separate laboratory experiments in which prior plant damage and the presence and genetic strain of fungi will be manipulated. Biomass gain, consumption and survival will be monitored in caterpillars, while reproduction of aphids will be assessed. Nutritional chemicals (protein nitrogen) and potential allelochemicals (ergots, lolines and peramine) in plants will also be assessed. In an additional experiment, potential indirect effects of endophytes on parasites of aphids will be assessed. Survival and growth of parasites will be assessed under host diets that vary with respect to plant and fungal genotype and the presence of fungal endophyte. Results will 1)assess effects of experimental combinations of plant and fungal genotypes on pest and beneficial insects, 2) shed light on mechanisms by which endophytes influence plant-insect interactions and 3) assist efforts to manage endophytes in pasture systems.

9901501 Plant Polypyrimidine Binding Proteins as Chromatin Derepressors

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New Investigator Award/Strengthening Award; Grant 99-35301-8023; $118,350; 2 Years

Plant and animal cells produce a form of histone H3 protein that assists the maintenance of chromatin structure in differentiated cells. Genes for such histone H3 variants in plants are constitutively expressed despite the presence of typical cell cycle control promoter elements. Phylogenetic gene analysis suggests that polypyrimidine (PPY) sequence elements are required for the constitutive expression. They are located among the promoter elements, in the untranslated regions (UTRs) and in the introns. We hypothesize that they bind proteins similar to the *Drosophila* GAGA factors, which cause chromatin derepression by preventing nucleosome assembly. In *Arabidopsis*, intron insertion with these elements caused strong increase in reporter gene expression and loss of the cell cycle control of a histone promoter. An alfalfa histone H3 promoter, which contains no intron in the 5' UTR but instead similar PPY motifs, conferred high, constitutive reporter gene expression . Gel shift analyses detected specific interactions between plant nuclear proteins and PPY motifs in the 5' untranslated and intragenic regions of the alfalfa gene. This project has the specific aim to confirm whether PPY elements cause high, constitutive gene expression in transgenic plants. Secondly, PPY-binding proteins will be purified from plants, sequenced and related cDNA clones will be isolated. Transgene silencing by heterochromatin is a major problem in plant biotechnology. The use of PPY sequences as natural gene de-repressors in the promoters and other parts of plant genes will be an invaluable tool to prevent gene inactivation and to maintain high expression of a desired gene throughout plant generations.

9903816 Phytoremediation/GEM Systems for Atrazine Remediation and Water Resource Protection

Burken, J.G.
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Seed Grant; Grant 99-35106-8244; $44,110; 2 Years

The purpose of this project is to develop and evaluate a unique plant/bacterial system designed to remediate the problematic organic contaminants, initially targeting atrazine. The overall goal is to create a new paradigm for organic contaminant remediation by creating a broadly-applicable technique that will inexpensively degrade contaminates *in-situ*. This technique could be used to remediate atrazine at individual farms, non-point sources, and agricultural dealerships, where other techniques are prohibitively expensive. This work is a novel collaboration that combines rhizoremediation and phytoremediation to create more effective remediation schemes for pesticides and chlorinated solvents. It is the first use of genetically-engineered microbes (GEMs) for the degradation of atrazine in the rhizosphere (there currently is no cost-effective remediation technique for atrazine). The project will also work to increase the knowledge base of the plant/GEM interaction, potentially opening door to other uses of GEMSs in agriculture.

If this project is successfully completed further research finding will be sought to deliver a new technology for cleaning atrazine-contaminated sites and to protect groundwater. These activities will help to limit the environmental impact of agrichemical leaks and spills. The overall result will be a technology that is useful and publicly embraced. Such a technology will increase the public acceptance of agri-chemical dealerships and display their efforts to preserve our environment.

9903641 Acquisition of Modern Cryostat for Agri-Research
Ainsworth, A.J.
Mississippi State University; College of Veterinary Medicine; Mississippi State, MS 39762
Equipment Grant; Grant 99-35208-8198; $8,975; 1 Year

The goal of this Equipment Grant is to purchase a modern, up-to-date cryostat for cutting tissue sections of frozen specimens. This piece of equipment is necessary because tissue staining methods requiring frozen tissue sections are commonly performed by scientists at the College of Veterinary Medicine. In addition, some specialized staining reagents will only work on frozen tissue sections, as opposed to standard paraffin tissue sections. The cryostat will be used primarily by veterinary medical researchers, especially those working in aquaculture. In addition, the advanced capability afforded by this new cryostat will also improve CVM's effectiveness in diagnostic investigations and disease surveillance thus facilitating the transfer of research advances to veterinarians, diagnostic laboratories, extension and agricultural producers.

9903680 Insect Neuropeptide Gene for Expression in Baculovirus Vectors
Ma, P.W.K.
Mississippi State University; Department of Entomology and Plant Pathology; Mississippi State, MS 39762
Equipment Grant; Grant 99-35311-8376; $24,600; 1 Year

The overall project has two objectives: 1) To study the structure and function of insect neuropeptides, and 2) To improve crop quality and productivity and the sustainability of agriculture by developing novel methods of controlling insect pest. The proposed research concentrates on a neuropeptide. Hez-PBAN (pheromone biosynthesis activating neuropeptide), that is found in the corn earworm, *Helicoverpa zea.* Hez-PBAN is encoded in a multifunctional gene. Its gene products, five peptides including Hez-PBAN, are involved in regulating sex pheromone production in moths, cuticular melanizalion in larvae, embryonic diapause as well as other physiological functions. Expression of the Hez-PBAN gene is localized in three distinct cell clusters in the subesophageal ganglion. Hez-PBAN gene is expressed in larval, pupal and adult stages. The present proposal is to focus on characterizing the peptides that are released from the Hez-PBAN precursor in individual cell clusters in the subesophageal ganglion, and in the blood of both adults and larval stages. The functional roles of each of the three subesophageal ganglion cell clusters will he studied by surgical ablation. This research will explore the combinations of peptides derived from the Hez-PBAN precursor that are responsible for regulating sex pheromone production in female adult and cuticular melanization in larvae. The information gained wil1 be used later to develop an optimized gene construct to be inserted into baculovirus vectors for insect pest control purposes.

9903667 Acquisition of a Gene Gun to Evaluate Function and Antigenicity of Gene Products
Hanson, L.A.
Mississippi State University; College of Veterinary Medicine; Mississippi State, MS 39762
Equipment Grant; Grant 99-35208-8545; $16,995; 1 Year

Mississippi's leading animal production industries are poultry and channel catfish. Because both industries use intensive production systems, infectious disease outbreaks can cause production failure in a short period. Research at the College of Veterinary Medicine, Mississippi State University has focused on the immune systems of chickens and catfish and on the development of useful vaccines for these industries. Proteins expressed from genes of pathogens are being evaluated for their vaccine potential. Also, we produce antibodies for use in diagnostic tests and for use in research to evaluate protein expression. The gene gun will be used to vaccinate research animals. The gene gun is a tool that can discharge DNA coated gold microprojectiles into the skin of research animals. This DNA is expressed into proteins and the animal develops an immune response to the protein. Gene gun based DNA vaccination is 100 times more efficient in inducing a strong immune response than syringe injection. We will use the gene gun to efficiently produce antibodies to the products of cloned genes in mice and rabbits. These antibodies will be used to detect the protein products of the genes in research, and to develop sensitive diagnostic assays. We will also use the gene gun to vaccinate catfish and chickens with DNA containing genes from pathogens to determine which genes code for proteins that induce protective immunity against disease.

9903713 Epifluorescence Microscope for Insect Pathology and Entomology Research
Inglis, D.G.
Mississippi State University; Department of Entomology and Plant Pathology; Mississippi State, MS 39762
Equipment Grant; Grant 99-35311-8406; $16,804; 1 Year

Every year, insects and mites cause billions of dollars in damage to crops and incite the use of potentially harmful pesticides. With a growing awareness of the potential harm from conventional pesticides (insecticide resistance, environmental damage, human health risks), the need for alternatives for pest management is pressing. One of the most promising potential means of controlling pests is through biological control by natural enemies, including use of pathogens (microbes that cause disease in insects) or predators and parasites (which attack crop damaging insects). The insect pathology program at Mississippi State University (MSU) is committed to both, the discovery of pathogens that can be used directly against crop pests and support for programs in mass production of non-microbial natural enemies of crop pests that

must be reared under conditions where pathogens of the beneficial insects are eliminated or reduced. In both cases, a thorough understanding of the pathogenic microbes and their interaction with insects is absolutely essential. One of the most important, modern tools in development of such an understanding of the pathogen-insect relationship is the epifluorescence microscope. The microscope system whose purchase is proposed here will be used for discovery and characterization of novel insect pathogens, characterization of tissue damage from these pathogens, development of biorational strategies, and teaching insect pathology students state of the art microscopy techniques. This expanded base of information and resultant technology transfer from using this equipment will benefit agriculture in Mississippi and the United States by helping to improve technologies for insect pest management.

9902343 Role of *Edwardsiella ictaluri* Lipopolysaccharide in Disease Pathogenesis

Lawrence, M.L.
Mississippi State University; College of Veterinary Medicine; Mississippi State, MS 39762
Strengthening Award; Grant 99-35204-7679; $125,000; 2 Years

Channel catfish farming has become an important agricultural industry, with over 5.6 million pounds of farm-raised catfish processed in the U.S. during 1998. The most serious disease affecting farm-raised channel catfish is enteric septicemia of catfish (ESC), which is caused by *Edwardsiella ictaluri.* At the Fish Diagnostic Laboratory in Stoneville, Mississippi, which serves an area containing the largest number of catfish farms in the U.S., *Edwardsiella ictaluri* was isolated from 41.2% of catfish cases submitted for disease diagnosis in 1998. The purpose of this study is to gain a better understanding of the outer polysaccharide coat of *Edwardsiella ictaluri* and the role it plays in causing disease. The polysaccharide coat of *Edwardsiella ictaluri is* composed of long chains of repeating sugar subunits, termed lipopolysaccharide. In other bacterial pathogens, lipopolysaccharide plays an essential role in disease by forming a protective coat around the bacteria, allowing them to resist certain components of the host immune response. Previously published research on *Edwardsiella ictaluri* has already provided evidence that its polysaccharide coat is important in the disease process. In this study, we will clarify the role of *Edwardsiella ictaluri* lipopolysaccharide in the disease process by determining how it affects specific essential components of the channel catfish immune response. We will also study how lipopolysaccharide affects the overall disease process in channel catfish. By gaining a better understanding of the role *Edwardsiella ictaluri* lipopolysaccharide plays in disease, we may be able to develop an improved vaccine or a new treatment for ESC.

9901739 Development of *Podophyllum peltatum* as an Alternative Crop for Small Farmers

Moraes, R. M.; Burandt, C.L.; Khan, I.A.
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Strengthening Award; Grant 99-35504-8261; $140,000; 3 Years

Etoposide and teniposide are semi-synthetic anticancer drugs for which the starting compound is podophyllotoxin, a natural lignan found in *Podophyllum* species. Etoposide has been used for the treatment of lung and testicular cancer, and teniposide is used to treat leukemia. In order for a therapy involving the use of natural products to be successful a reliable drug supply is needed. This is a critical issue for natural products, derived from collections of wild populations. The commercial source of podophyllotoxin is *Podophyllum emodii* Wall. (Syn. P. *hexandrum* Royale), a species native to the Himalayas, which was declared in danger of extinction during the 1989 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). As a result, the export of the raw material of *P. emodii* was prohibited. The resin, however, is still being exported, a practice that contributes to the disappearance of the species. An alternative source could be the North American species *Podophyllum peltatum* L. (mayapple); therefore a survey of the United States' population was done. We have identified and selected some promising accessions. These are high yielding plants, which have been propagated to establish a variety repository. The objectives of our study are:1)to confirm elite genotypes for podophyllotoxin production; 2) to produce healthy propagules and evaluate somaclonal variation; and 3) to determine the effects of pre- and post-harvest treatments on podophyllotoxin yield. We expect our research to have a positive impact on the supply of podophyllotoxin for cancer patients and for mayapple to become an alternative source of income for American farmers.

9903676 Tougaloo College Rural Development Initiative

Rozman, S.L.; Abdelrahman, K.A., Mohamed, E.A.
Tougaloo College; Social Science Division; Tougaloo, MS 39174
Seed Grant; Grant 99-35402-8302; $50,000; 2 Years

We will be doing preparatory work for a longitudinal study of a representative sample of African-American farmers in Mississippi who have settled their racial discrimination lawsuit with the United States Department of Agriculture and are in the process of receiving compensation for losses they experienced.

The seed grant will enable us to: (1) identify a rural population that we will target for study; (2) develop a database for empirical, interdisciplinary research; (3) strengthen the skills of the faculty investigators-and later, their students-for

engaging in rural development research; and (4) establish contact with local agencies and organizations which serve the targeted population.

We will be exploring (1) the effect of the recent settlement on the farm family's management of their social, financial, and civic responsibilities; (2) the impact of the prior discrimination on the family unit and the family's strategy for coping with the impact; (3) patterns of unusual or abnormal behavior exhibited by farmers that can be directly attributed to the discrimination they experienced; (4) identification of the social infrastructure needed by the Mississippi farmers, in preparation for the new millennium, that will provide them with a reasonable chance of success in the agricultural arena.

Our project is significant to agriculture since it will position the principal investigators to serve as facilitators for communication among the targeted farmers and between the farmers and agencies/organizations relevant to the farmers' interests. We will also strengthen our ability to evaluate ongoing rural development programs and initiatives in addition to contributing to the discussion related to new policies and initiatives.

9903586 Microbial and Surface Area Effects on Surface Water Transport of Dissolved Metals

Shiller, A.M.
University of Southern Mississippi; Department of Marine Science; Stennis Space Center, MS 39529

Seed Grant; Grant 99-35106-8604; $50,000; 2 Years

The goal of this project is to develop a better understanding of the processes controlling dissolved concentrations of metals such as lead and zinc in surface waters. These metals are strongly adsorbed onto the surfaces of suspended particles. Among the most important particulate adsorbing surfaces in natural waters are manganese oxides. Previous work has shown that these oxides can be produced by manganese oxidizing bacteria which are ubiquitous in the environment. Furthermore, there is evidence that seasonal changes in water temperature affect the rate of microbial manganese oxidation. Other work has shown that microbially produced manganese oxides have a far greater specific surface area than inorganically produced manganese oxides. These observations lead to the central hypothesis of the work proposed here: seasonal variability in dissolved lead and zinc concentrations in rivers can be caused by seasonal changes in microbial manganese oxidizing activity in the water. Furthermore, it is anticipated that agricultural activities will facilitate the mobilization of soil microbiota into local surface waters and thus we expect to see a greater microbial surface area effect in streams affected by agricultural activities than in streams not so affected. The work proposed here is of additional relevance to USDA because agricultural activities (both land management and water usage) can affect the transport of potentially toxic metals through processes such as soil erosion and runoff of irrigation waters. Understanding the mechanisms that control dissolved concentrations of potentially toxic metals in surface waters is a fundamental step in understanding how to alleviate problems that may arise from agricultural activities. The work will involve field and laboratory studies of metal cycling in the lower Mississippi River and tributaries of the Pearl River (MS).

9903600 Modulation of Bovine Neutrophil Function by Cell-Cell Interactions and Extracellular Matrix Proteins

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Seed Grant; Grant 99-35208-8401; $49,989; 2 Years

Livestock, like humans, are constantly exposed to potentially pathogenic microorganisms. Far from being powerless, animals have complex host defense mechanisms. One of the front line defenders is the white blood cell known as the neutrophil. The neutrophil itself has a complex array of antimicrobial devices. With the appropriate stimuli, neutrophils can migrate to sites of infection, alter their cell surface to facilitate interactions with other cells, produce molecules that can directly kill microorganisms, release reactive oxygen compounds, and phagocytize bacteria and fungi. Although neutrophil function is essential for defense, their continued presence at sites of infection can be harmful to the animal and damage host tissue. An example of this is bovine mastitis, where neutrophils provide necessary defense against bacteria invading the udder; however, lingering elevated numbers of neutrophils can damage the udder and reduce milk quality and yield. The economic consequences of mastitis are substantial; annual losses in the United States alone are estimated at $2 billion. For any potential therapy to be useful, neutrophil function would need to be modified discretely and specifically, reducing tissue damage without compromising host defense. Neutrophil function *in vivo* is complex and modulated by many endogenous factors. Compounds released from neighboring cells, or direct contact with those cells can both accelerate or reduce neutrophil actions. I propose to examine one set of these interactions: whether neighboring blood vessel cells, mammary epithelial (udder) cells, or extracellular matrix proteins modulate the response of bovine neutrophils to selected compounds which normally induce inflammatory responses from neutrophils.

9903649 Equipment Grant for a Quantitative Polymerase Chain Reaction Instrument

Burgess, D.; Hardy, M.E.; Jutila, M.A.; Pascual, D.W.; White, M.
Montana State University; Department of Veterinary Molecular Biology; Bozeman, MT 59717

Equipment Grant; Grant 99-35208-8197; $25,000; 1 Year

This project is to support the purchase of a high throughput instrument for use in determining coding messages for mammalian and microbial proteins [messenger RNA levels; mRNA]. Several projects in our department examine host leukocyte responses to infection/inflammation and/or microbe responses during host/parasite interactions [e.g., intracellular microbial growth and development] in the broad context of livestock diseases. These processes result in changes in the mRNA levels of proteins such as cytokines in leukocytes ("immunological hormones"), surface proteins such as adhesins on leukocytes and microbial proteins important in virulence and pathogenesis. Changes in mRNAs precede changes in the proteins and can, therefore, be used to determine when such proteins are induced in host and microbial cells. This information will lead to a better understanding of processes such as host inflammatory and immune responses to pathogens, the pathogenesis of disease caused by these microorganisms and the developmental biology of microbial pathogens of livestock.

9901742 Genetics and Biochemistry of Wheat Grain Hardness Genes

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New Investigator Award/Strengthening Award; Grant 99-35301-7764; $120,000; 2 Years

Grain hardness in wheat is a primary factor of wheat flour quality. Grain texture or hardness is a function of the physical texture of the endosperm, and influences such quality parameters as milling energy, particle size, starch damage, baking quality, and water absorption. These quality parameters in turn, strongly impact the commercial usage and value of wheat. Hard wheat flour is used primarily for bread, while soft flour is used primarily in cookies, cakes, and pastries. A single locus, *Hardness (Ha),* controls the difference between hard and soft wheat. This gene difference is marked by soft *(Ha)* or hard (*ha*) textured endosperm and large (*Ha*) or small (*ha*) amounts of the marker protein friabilin on the surface of starch granules. Sequence or transcriptional alterations in the two genes that encode friabilin, *puroindoline a* and *b (pinA* and *pinB,* respectively), are inseparably linked to the wheat *Hardness* locus in wheat that is hard textured. The unbroken linkage between putative mutations in these two genes and hard textured endosperm suggests that they together comprise the *Hardness* gene. Additionally, the putative mutation in *pinB,* a simple glycine to serine change is common in hard wheats that are softer than those with the *pinA* alteration, a null mutation. The data suggest that the *puroindoline* genes act together, to effect softness. The experiments proposed are directed at current shortcomings in our knowledge of endosperm grain texture and the project aims to conclusively identify the genes controlling endosperm texture in wheat. Specifically, the objectives of this project are to conclusively identify the *pinA* and *pinB* genes as functional components of the *hardness* gene using transgenic plant technology. The second objective will quantify flour quality difference between hard wheats containing an alteration in *pinB* versus those devoid of the PINA protein.

9903508 Leukocyte Adhesion Protein Expression and Cytokine Production in Mastitis

Soltys, J.; Quinn, M.T.
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Seed Grant; Grant 99-35208-8623; $49,988; 2 Years

Mastitis is a complex disease resulting from infection of the mammary gland by a number of different types of bacteria. During mastitis white blood cells or leukocytes respond to infection of the mammary gland by migrating into the udder, resulting in inflammation. These responses are necessary for the early stages of host defense; however, prolonged inflammation can result in tissue damage. Lingering elevated numbers of leukocytes in the udder can also reduce the quality and yield of the milk. Although enhanced udder hygiene programs along with a better understanding of bacterial pathogenesis have helped in the reduction of mastitis, mastitis is still the most common and costly disease affecting the dairy industry. Currently, not many effective treatments exist for reducing the prevalence of mastitis; however, this is likely due to a lack of understanding of the mastitis process. Clearly, the development of potentially effective therapies for preventing mastitis requires a better understanding of the processes involved in this disease. More specifically, a better understanding of the role of the bovine immune system in combating mastitis infection will be essential.

In this Seed Grant, we propose to investigate how bacterial toxins regulate adhesion molecule expression and cytokine responses by bovine leukocytes and if beta-glucan can modify these responses. By fully characterizing these responses, we may be able to develop agents that could be used in the future as therapies for enhancing host defense in the udder and reducing the prevalence of mastitis.

9903721 Equipment for the Physical Location of Specific Sequences through In Situ Hybridization
Sherman, J.D.; Talbert, L.E.
Montana State University; Department of Plant Sciences; Bozeman, MT 59717.
Equipment Grant; Grant 99-35311-8375; $18,987; 1 Year

Wheat is the major crop in the Northern Great Plains of the United States. Production is often limited, and quality is negatively affected, by a range of insects, diseases, and environmental stresses. Barley has genes that confer resistance to many of these stresses. Our goal is to transfer desirable genes from barley into wheat. This project requires manipulation of the wheat and barley chromosomes, and the use of specialized equipment. In particular, we will purchase a thermocycler for conducting PCR, a spectrophotometer for measuring DNA concentration ,and a microscope for viewing plant chromosomes. Successful completion of the research will allow more sustainable and profitable wheat production in the Northern Great Plains.

9902245 Bull-cow Interactions Attenuate the Cow-calf Bond Regulating Anestrus
Berardinelli, J.G.
Montana State University; Department of Animal and Range Sciences; Bozeman, MT 59717-2900
Strengthening Award; Grant 99-35203-7932; $95,000; 3 Years

The goal of this research is to determine how the presence of bulls reduces the period of non-reproductive activity of cows nursing calves. We will evaluate the novel hypothesis that bulls and cows form a "bonded" relationship that either reduces the negative effect of nursing calves (the cow-calf bond), or directly stimulates the brain centers responsible for initiating the onset of reproductive activity. In these experiments we will determine if the presence of bulls decreases the cow-calf bond allowing establishment of a bull-cow "bond": accelerating early return to reproductive activity. We will then determine whether the bull produces pheromones (chemical agents produced by individuals that alter the behavior of others of the same species) that in and of themselves are sufficient to "break" the cow-calf bond or require the behavioral aspects of bulls to do so. Lastly we will attempt to show that a cow-bull bond is established when calves become more "independent" of dams, when the bull effect becomes evident in cows, by substituting an "alien" bull for a "familiar" bull (exposed to cows soon after calving). Results of these studies should support the novel idea that bulls reduce the postpartum non-reproductive period by forming a social or behavioral bond with cows, when calves become less dependent upon dams. If this idea proves to be correct, it could substantially improve our understanding of the important role of bulls in the reproductive activity of cows. With such understanding, new cost-effective management strategies may be developed and tested that employ bulls to enhance the reproductive efficiency and sustainability of beef cattle production in the United States.

9901709 Development of an Efficient and Scalable Synthetic Route to D-Glucaric Acid
Kiely, D.
University of Montana; Shafizadeh Rocky Mountain Center for Wood and Carbohydrate Chemistry; Missoula, MT 59812
Strengthening Award; Grant 99-35504-8262; $89,605; 2 Years

The long term goal of this research is to develop new value added products from glucaric acid, a six carbon carbohydrate diacid derived from glucose (dextrose), a major commercial agrochemical from corn starch hydrolysis (corn refining industry). We envision that when a commercial process for producing glucaric becomes available, glucaric acid will become a large scale "stand alone" chemical with a wide range of commercial applications, including those in the food, diet supplement, detergent, cosmetic, and pharmaceutical industries. In addition glucaric acid is the principal renewable resource "building block" for a number of novel synthetic polymers (polyhydroxypolyamides) developed in the PI's lab. These materials are targeted as both biodegradable functional materials (e.g., for use as environmentally friendly components in various manufacturing processes) and building materials (e.g., for use in short term biodegradable plastics applications). The specific goal of this project is to establish an efficient laboratory procedure for the chemical conversion of glucose to glucaric acid that will quickly lead to a pilot plant scale and then industrial scale process. Funding for this project will allow us to evaluate, and hopefully develop a sound lab scale version of the conversion step as part of a projected commercial process for glucaric acid.

9903506 Phenolics from Larch Needles
Ponder, G.
University of Montana; Center for Wood and Carbohydrate Chemistry; Missoula, MT 59812
Seed Grant; Grant 99-35501-8660; $49,224; 2 Years

Increasing awareness of the nutritional benefits and pharmacological potential of flavonoids points to the need to investigate previously untapped sources of these and related natural phenolic compounds. Larch needles, which are shed in the fall, are one such source, and preliminary results suggest that they may be a good source. Moreover, previous work indicates the likelihood that some flavonoids in larch needles are pharmacologically active. Therefore, as a first step toward investigating larch needles as a possible source of commercially valuable phenolics, these needles will be collected at different times of year and analyzed for their phenolics content. This will involve extraction of the needles with polar

solvents, followed by sub-fractionation of the extracts by liquid-liquid extractions. Column chromatography will be used to achieve further sub-fractionations. The types and amounts of phenolics present in the fractions will be determined primarily by means of high-performance liquid chromatography coupled with diode array ultraviolet spectroscopy. Low-pressure preparative chromatography and nuclear magnetic resonance spectroscopy will also be important tools for isolating and identifying specific compounds. The work will also include assays aimed at identifying biologically active mixtures or specific compounds. Thus, various fractions will be screened for possible enzyme suppression effects. These screenings will be conducted by an external lab. The presence of such biologically active compounds in needles of larch, or of other types of conifers, could lead to marketable products and contribute to value-added opportunities in the forest products industry.

9903513 Which Isomer Conjugated Linoleic Acid Attenuates Preadipocyte Growth?

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Seed Grant; Grant 99-35207-8622; $50,000; 2 Years

The incidence of obesity or excess adipose tissue is highly prevalent in the US. Dietary lipids contribute significantly to excess energy intake, which leads to the development of obesity and related diseases. Surprisingly, two recent studies found that mice fed low levels of conjugated linoleic acid (CLA), an unsaturated fatty acid found in beef and dairy foods (important products from the U.S. agricultural industry), had less body fat than control mice. These studies suggest that the inclusion of CLA in the diet may help prevent or attenuate the development of obesity. However, the mechanism by which CLA reduced body fat in these mice and how CLA influences (pre)adipocyte growth are not known. Furthermore, the potential antiobesity properties of CLA in humans are unknown. We have preliminary data demonstrating that mixed isomers of CLA attenuate the differentiation of murine 3T3-L1 preadipocytes into mature adipocytes. Therefore, the objectives of this USDA NRI Seed Grant are to determine: 1) which CLA isomer attenuates 3T3-L1 preadipocyte differentiation most effectively; and 2) whether the CLA isomer induces either cell cycle arrest or apoptosis. The results of these studies will provide us with critical data to design future studies to examine: 1) specific antiobesity mechanisms of action of CLA; and 2) to determine whether CLA attenuates the differentiation of human (pre)adipocytes, and by what mechanisms. If these data demonstrate antiobesity actions of CLA, future animal and clinical trials with CLA-containing foods or supplements may be warranted.

9903605 Knowledge and Use of Dietary Supplements among Women of Childbearing Age

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Seed Grant; Grant 99-35207-8519; $49,988; 2 Years

Dietary supplements are used by more than 50% of the U.S. population according to recent national surveys. Women are reportedly frequent users of dietary supplements. Most research about dietary supplements has addressed the characteristics of supplement users or the relationship between nutrient adequacy and supplement use. Little research has investigated consumers' knowledge of appropriate supplement use or the processes consumers use to purchase dietary supplements. Models of health behavior state that individuals will engage in preventive health behavior if they believe they are susceptible to ill health, if the consequences of ill health are severe, and if the benefits of taking action outweigh the barriers. Therefore, the purpose of this study among women of childbearing age (aged 25-45 years) who use at least one dietary supplement four times per week is to evaluate why they consume supplement(s), the decision-making processes used to purchase supplements, and their comprehension of nutrition and supplement label information. Fifty women will complete a telephone screening interview to determine subject eligibility, a written mail questionnaire about supplement use, and an individual point-of-purchase shopping interview. The interviews will be analyzed using an integrated qualitative and quantitative approach. Data from the written mail questionnaire will be used to evaluate knowledge of nutrition and supplement label information and the degree constructs of preventive health behavior predict supplement use. This research can be used to update federal dietary recommendations about nutrient adequacy and supplement use targeted to women of childbearing age.

9902151 Intercropping as a Disease Management Tool for Early Leaf Spot of Peanut

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Strengthening Award; Grant 99-35316-8007; $90,000; 2 Years

Early leaf spot of peanuts (ELS), caused by the fungus *Cercospora arachidicola, is* a devastating disease worldwide, with few control options available. Intercropping, the cultivation of multiple crop species in close association often employed in subsistence agriculture, will be evaluated for its potential as a non-chemical, biologically-based disease management strategy in a modern industrialized production setting. Peanuts inter-cropped with corn will be our model system, but the approach engendered here could be applied to numerous and diverse other systems, both large-scale mechanized and small-scale manual, in the U. S. and abroad. This goal will be accomplished through three specific

objectives. First, we will quantify ELS incidence and crop yield throughout two seasons in field plots containing peanut monocrops, intimate intercrops (alternating rows of corn and peanuts), and strip intercrops (4 rows corn alternating with 4 rows peanuts). Second, we will compare disease dispersal patterns among these treatments. Third, we will evaluate the impact of proximity to corn on disease development after dispersal, particularly as influenced by changes in microclimate, at a separate location. The first goal has immediate application; the second goal will improve our understanding of the mechanisms at work and help generalize to other crop-disease systems. Preliminary data suggests that corn can reduce ELS severity on peanuts. If compatible with mechanization, intercropping could be readily adopted by many farmers as a low-input cultural control in this case and others, particularly where few alternatives to chemical disease management exist. This will enhance the long-term sustainability of U. S. agriculture.

9903457 Building Sustainable Communities: Natural Resource Management Tools for Local Communities Using the Southern Appalachian Assessment Data Set

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Sabbatical Grant; Grant 99-35402-8286; $50,264; 1 Year

The Southern Appalachian Assessment Area is recognized for its significant level of natural assets that create a rural character and provide a quality of life that is attractive to its current and potential residents and businesses. Development in the region is occurring at rates similar to other areas containing significant natural assets. This project will develop a practical management approach for local government and other local community based organizations to address the growth and environmental issues they face. In order to make choices about our natural and environmental resources we need to know what we have and how these resources link to our economic health. Resource inventories provide the information we need to know what we have. This project will ultimately develop a practical method for understanding the links between a community's natural asset base and it's economic well being using Geographic Information System technology with the Southern Appalachian Assessment data set and additional economic data.

9903632 Mutagenesis of the Increased Serum Survival Gene (*iss*) to Determine its Role in the Virulence of Avian *Escherichia coli*

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Seed Grant; Grant 99-35208-8129; $50,000; 2 Years

Colibacillosis is a major detriment to the poultry industry in this country, yet few studies have been directed toward improving colibacillosis control. Previous studies in our lab have suggested that complement resistance may be a major virulence factor in avian *E. coli*. Recently, *iss*, the increased serum survival gene, was cloned from a virulent, complement-resistant avian *E. coli* isolate and its identity confirmed by sequence analysis. *Iss*, which encodes an outer membrane protein, is strongly associated with virulence among avian *E. coli* isolates. Of the *E. coli* isolates examined, *iss* was detected in 78% of the isolates from sick birds and 19% of the isolates from apparently healthy birds, indicating that it might be a reasonable target for new colibacillosis control strategies. In this study, *iss'* role in virulence and complement-resistance of avian *E. coli* will be better defined in order to assess its potential as a target of colibacillosis control strategies. Using amplification and site specific cloning techniques, the *iss* gene will be deleted from the genome of a virulent, complement-resistant avian *E. coli* isolate. Differences in the complement resistance and virulence of the wild-type parent and its isogenic *iss* mutant will be detected with flow cytometry and an embryo lethality assay. Such studies will better define the virulence mechanisms of avian *E. coli*. Once it is known how *iss* allows *E. coli* to evade the effects of host complement, future *iss*-based control strategies can be designed and thus reduce poultry losses due to *E. coli* infections.

9903452 Acqusition of a High-speed Centrifuge

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Equipment Grant; Grant 99-35208-8544; $25,000; 1 Year

The mechanism by which nitrogen fixation (conversion of atomospheric nitrogen to bioavailable ammonia) is regulated in accordance with the amount of oxygen present is not well understood. This project investigates the means by which a heme-containing protein called FixL regulates transcription of the genes necessary for nitrogen fixation in response to the amount of oxygen present in the cells where fixation occurs. The funds from this grant will be used to purchase a high-speed centrifuge to facilitate the isolation and purification of the oxygen sensing protein FixL for spectroscopic investigation in our laboratory.

9902510 Construction of an Ordered, Complete Genomic Library for the X-disease Phytoplasma

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Strengthening Award; Grant 99-35303-854; $100,000; 2 Years

Phytoplasmas are a group of cell wall-less, insect-transmitted prokaryotes. They systemically parasitize plants in phloem tissues and cause diseases in more than 300 species worldwide, resulting in multi-billion dollar losses each year in the U.S. alone. To date, there are no effective measures to control or cure these diseases because nearly all key issues related to phytoplasmas have not been resolved. The objective of this proposal is to construct, the first of its kind, an ordered, single-copied, complete genomic library for the X-disease phytoplasma. We have developed/modified the "Long-Distance-PCR Genome Walking" strategy, a cycle of primer design, PCR amplification, end sequencing, and new primer design. We have "walked" six steps both upstream and down stream from the tRNA"e gene in the 16S/23S rRNA spacer region. The largest amplified fragment in each direction and each step have been saved/cataloged as a part of the genomic library. We will continue "walking" through the genome of the X-disease phytoplasma, thus establishing a single-copied, complete genomic library with known orders. Our long-term goals are to completely sequence the entire genome of the X-disease phytoplasma, and to utilize the sequence data to study critical questions in phytoplasmology, such as culturability, classifications and phylogeny, host/parasite interactions, genetic variation. Better understanding of phytoplasma's evolution and host and vector interactions will eventually lead to developing strategies to control or cure the X-disease and other phytoplasma-associated diseases, thus enhancing the competitiveness of U.S. agriculture /horticulture /forestry in the increasingly competitive world market.

9903527 Fine Structure Mapping of Loci Affecting Nuclear-Cytoplasmic Interaction in Tetraploid Wheat

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Equipment Grant; Grant 99-35311-8252; $50,000; 2 Years

Genes transferred from wild species for pest resistance alone have contributed substantially to the agronomic improvement of important crops, including wheat. Sterility in hybrids remains one of the major obstacles to alien gene transfer, and genes producing nuclear-cytoplasmic (NC) compatibility are directly or indirectly involved. A better understanding of NC interactions would permit wider usage of wild species and more efficient gene introgression. Populations segregating for two of the genes involved in NC interaction *(scs* (species cytoplasm specific) and *Vi* (vitality)) in durum wheat have been generated. We plan to use these populations to identify tightly linked molecular markers and determine the effect of these genes on phenotype of normal durum lines. Linked molecular markers will be invaluable in efficient deployment of *scs* and *Vi* leading to the development of the "two-gene" hybrid seed production system in wheat, as well as, cloning and detailed characterization of the mechanisms involved. It is believed that in certain combinations these NC compatibility genes improve fertility beyond the level of current durum cultivars. Development and identification of homozygous lines for *scs* and Vi through this project will enable us to determine the effect of these genes on plant fertility. A better understanding of these interactions has direct applications to plant breeding and crop improvement.

9903663 Acquisition of Assisted Reproductive Technology Equipment

Grazul-Bilska, A.T.; Redmer, D.A.; Reynolds, L.P.; Tilton, J.E.

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Equipment Grant; Grant 99-35208-8128; $25,000; 1 Year

Funds are requested for the purchase of equipment for research in animal embryology and assisted reproductive technology (ART). This equipment will help to establish a new animal embryology/ART facility at NDSU. The proposed studies on reproductive processes in domestic animals will expand research and teaching opportunities for faculty, staff and students in the rapidly developing areas of animal biotechnology, embryology and ART. Once established, the animal embryology/ART laboratory will support a wide variety of research projects in the basic and applied sciences with the following long-term goals: 1) control of ovarian function; 2) *in vitro* fertilization (IVF) and embryo manipulations; 3) investigation of the role of cellular interactions in embryo development; 4) production of transgenic animals; 5) marker-assisted genetic selection; and 6) development of preimplantation diagnostic tests. The specific objectives of the proposed research projects are to: 1) optimize and apply laparoscopy techniques for retrieval of oocytes; 2) perform IVF and evaluate fertility rate, blastocyst development, and success of cryopreservation of embryos; and 3) evaluate the role of cellular interactions in embryo development in sheep and/or pigs.

The proposed experiments may lead to practical and(or) commercial applications. There is a growing demand from farm animal producers for modern methods to improve reproductive efficiency and lower the cost of producing better quality animals. The modern embryology/ART methods provide the means to help producers apply modern biotechnologies. Modernization and/or adoption of existing techniques and discovery of new ones would have immediate benefits to animal production.

9903717 Automated DNA Sequencer for the University of New Hampshire

Kocher, T.

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Equipment Grant; Grant 99-35311-8117; $40,000; 1 Year

This award will support the purchase of an automated DNA sequencer for the University of New Hampshire. The instrument will be located in a central campus facility to provide DNA sequencing services to faculty in five academic departments. The sequencer will be used to construct genetic linkage maps of strawberries, mints and cultured fishes. Other projects will investigate the population genetics of forest trees and commercially important marine algae. The DNA sequencer will also be used to study the molecular biology of Arabidopsis, a plant which serves as an experimental model for crop plants. A final project will used DNA sequencing to identify toxic cyanobacterial blooms in public water supplies. The new DNA sequencer will provide improved capabilities to support our research programs over the next several years.

9903568 Organelle Genome Diversity in Fragaria

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Seed Grant; Grant 99-35311-8325; $49,998, 2 Years

Wild strawberry (Fragaria sp.) germplasm is an important resource for strawberry geneticists and breeders. However, to adequately address germplasm collection and conservation needs, a better understanding of species relationships within the genus Fragaria is needed. The long term goals of the project are to establish the phylogenetic relationships of the wild and cultivated strawberries, and to characterize nuclear-organelle genome interactions that hamper sexual hybridization between cultivated strawberries and their wild relatives. The specific project objectives and approaches are as follows.

l) A skeletal map of the Fragaria mitochondrial genome will be established via sequencing of random clones from a mitochondrial genome library (to be constructed).

2) A novel method called S1 nuclease analysis of long PCR (polymerase chain reaction) heteroduplexes will be used to detect phylogenetically informative DNA sequence variations called indels (insertions/deletions) in mitochondrial and chloroplast genomes.

3) Using the detected indel polymorphisms as genetic markers, the mode of chloroplast and mitochondrial genome inheritance (presumed to be maternal) *in Fragaria* will be determined.

9903737 Skin Structure and Pigment Defects in Summer Flounder

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Seed Grant; Grant 99-35208-8586; $49,989; 2 Years

Hatchery-reared flounders are often abnormally colored. Instead of the upper side becoming dark and the lower side losing pigmentation at metamorphosis, fish may turn white or mottled on top and retain dark areas underneath. The purpose of this project is to help determine the specific causes of malpigmentation in hatchery-reared flounder by examining the development of pigmentation in normally- and abnormally-colored skin. Identifying causes of pigment defects can help guide the search for a solution, and is critical to analyzing studies testing possible approaches. Both the market value of hatchery fish and future stock enhancement efforts will benefit from reducing the occurrence of malpigmentation, which renders fish both unattractive to consumers, and particularly vulnerable to predators in natural environments.

Understanding the origins of pigmentation defects depends on identifying differences between normal and abnormal skin and pigment cells. Specific structural differences may indicate possible developmental causes of malpigmentation, and thus, suggest potential remedies. In order to learn more about the structural basis and possible causes of pigment defects in hatchery-reared summer flounder, I will use several techniques to compare normally- and abnormally-pigmented skin areas. I will compare skin structure at the histological and ultrastructural level using antibody techniques together with light and electron microscopy. Two separate assays will be used to determine whether pigment cells disappear from light-colored areas through a process of self-destruction or intrinsically programmed cell death, and whether the mechanism is the same in normally unpigmented blind-side skin as in upper-side skin that shows abnormal loss of pigmentation.

9900975 Ecosystem Consequences of Globalized N Deposition - N Losses to Water and Air

McDowell, W.H.

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Strengthening Award; Grant 99-35101-8529; $100,000; 2 Years

The sources and distribution of anthropogenic nitrogen (N) are rapidly shifting from concentration in the more-industrialized temperate regions to a more global distribution. However, scientific understanding of the consequences of this anthropogenic nitrogen deposition, such as trace gas emissions and nitrate leaching, is based almost entirely on studies of N-limited systems carried out in the temperate zone. It seems likely that many, tropical ecosystems will respond quite differently to anthropogenic inputs of nitrogen due to differences in nutrient limitation. Tropical forests are hypothesized to be less able to retain anthropogenic nitrogen, and more likely to show immediate N losses in soil solution with increased N deposition. To evaluate this hypothesis, experimental plots will be established in the Luquillo Experimental Forest, Puerto Rico. Changes in soil solution chemistry will be measured in response to elevated N

inputs in two forest types. The experimental design consists of replicated plots receiving 150 kg/ha/yr N as NH_4NO_3, and a separate riparian experiment in which losses of N will be measured as shallow groundwater moves through the riparian zone. The results of this research project will have important implications for management and sustainability of tropical landscapes, and will provide fundamental information on the nature of the N retention process in terrestrial landscapes.

9903503 Ambient Air Quality Issues Related to Confined Animal Operations

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New Mexico State University, Carlsbad; Environmental Monitoring & Research Center; Carlsbad, NM 88220

Seed Grant; Grant 99-35208-8584; $49,976; 2 Years

The studies of air quality at animal feedlots supported by this award will bring together a multidisciplinary team of researchers who have expertise in resource engineering, atmospheric chemistry, meteorology, and microbiology. This is a preliminary sampling effort designed to evaluate the physical, chemical and microbial characteristics of airborne particulate matter from cattle feedlots in the southern High Plains. Aerosol particle samples will be collected on filters at strategic sampling points in and around one or more cattle feedlots in the southern High Plains. Analysis of the samples will produce data for (1) particulate mass loadings (the mass of particulate matter per cubic meter of air); (2) atmospheric concentrations of dust, major ions (nitrate, sulfate, chloride, fluoride, bromide, phosphate, calcium, magnesium and potassium), and a suite of trace metals; (3) bacterial and fungal viable counts, with some speciation of microorganisms; and (4) concentrations of organic nitrogen, nitrate, ammonium ion, and urea. Information obtained from these studies will provide a starting point needed for identifying and understanding the air quality concerns associated with cattle feedlot operations. The long-term goal for the project is to develop a standard proposal that will lead to an integrated, multidisciplinary research program designed to understand, predict, mitigate and communicate the health effects and socioeconomic implications of exposure to ambient particulate matter from feedlots.

9903829 Improved Detection of *Cryptosporidium parvam* in Soils

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Seed Grant; Grant 99-35106-8181; $49,197; 2 Years

This project will adapt an analytic technique used for testing biological samples (flow cytometry) for use in field studies of *Cryptosporidium parvam* oocysts in soil. We will compare the performance of flow cytometry with that of microscopy. Microscopy is the most widely used method of estimating numbers of oocysts in samples and evaluating their potential to infect new hosts.

Cryptosporidium parvam infected nearly 400,000 people in 1993, in the largest outbreak of waterborne disease in the history of the United States. Recent amendments to the Safe Drinking Water Act provide a framework for controlling sources of contamination to protect public water supplies and health. However, little is known about how pathogens like *Cryptosporidium parvam* move from soils to water. If we are to control potential sources of this pathogen, such as wastes from animal agriculture, we must have information about transport and survival.

Our limited ability to analyze soil samples has been a significant obstacle to gaining a good understanding of survival and transport. Even with recent improvements, the amount of time needed for microscopic analysis is the limiting factor to carrying out large-scale projects that could provide information about how microbes such as *Cryptosporidium* are moved by water in the soil environment. Flow cytometry has been explored as an alternative to microscopy and offers distinct advantages. Our project will compare precision and accuracy of estimates and limits of detection of the two methods applied for soil analysis.

9901082 The Effect of Harvester Ant Nests on Nitrogen Cycling in Arid Rangeland Soils

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New Investigator/Strengthening Award; Grant 99-35101-7834; $180,000; 3 Years

The goal of the proposed research is to estimate the effects of a common, soil-dwelling animal species, the harvester ant (*Pogonomyrmex barbatus*), on nitrogen cycling in arid rangeland soils. Often viewed as pests because of their seed eating habits and venomous sting, preliminary data suggest that harvester ants play a significant role in nutrient cycling. Ants concentrate organic debris in the vicinity of the nest, thereby creating islands rich in organic matter and mineral soil nutrients within the landscape. On the study site, harvester ant nests occupy only about 1% of the surface area of the landscape but we estimate that ant nest soils contain 9 - 14% of the total available soil nitrogen on the plot. We hypothesize that ant nests alter major N transformations and play an important role in controlling rangeland soil fertility. The influence of harvester ants on nutrient cycling at large spatial scales depends on the density, age and size of nests. The proposed research will combine information about how ants affect soil nutrient levels and nutrient cycling with information about ant population biology to estimate the effects of harvester ant populations on nitrogen cycling at large spatial scales.

9903566 Site-Directed Mutagenesis of Plant Carbonic Anhydrase
Rowlett, R.S.
Colgate University; Department of Chemistry; Hamilton, NY 13346
Seed Grant; Grant 99-35311-8651; $50,000; 2 Years

The goal of this project is to obtain detailed information concerning the molecular structure and the chemical mechanism of action of plant carbonic anhydrase, an enzyme which is considered to be essential for carbon fixation in higher plants. To accomplish this goal, the gene for a typical plant carbonic anhydrase will be isolated from Arabidopsis thaliana (mouse-ear cress) using recombinant DNA technology, allowing the large-scale production of highly purified enzyme for detailed structural analysis by X-ray crystallography, and precise study of the chemical mechanism by various kinetics methods. In addition, using established protein engineering methods of site-directed mutagenesis, the chemical structure of the enzyme will be specifically and selectively altered in order to better understand structure-function relationships in this important enzyme.

This research directly addresses a basic understanding of plant carbonic anhydrase, one of the critical molecular components of photosynthesis in higher plants, and the second most abundant enzyme in the photosynthesizing organelles of green plants. An understanding of the structure and function of this enzyme is essential for a complete understanding of the chemistry and efficiency of carbon fixation in higher plants, including those which are among the world's food crops.

9903454 Weight Management for Low-Income, Urban, African-American and Hispanic Women
Blocker, D.E.
Hunter College, CUNY; Nutrition and Food Science; New York, NY 10010
Equipment Grant; Grant 99-35207-8558; $25,000; 1 Year

The purpose of this one-year project is to systematically develop and implement a weight management intervention program specifically designed for low-income, urban African-American and Hispanic women. This is a population at extremely high risk for obesity and obesity-related health problems, and a population for which a paucity of data are available on successful weight management approaches. The goals of this proposed investigation are: a) to assess the health and nutritional status of low income African-American and Hispanic females, including the determination of body composition; and b) to use the data obtained in the assessment to develop and implement a weight management, physical activity, and wellness promotion intervention program. Body composition analysis is essential to the nutritional status assessment of the subjects, and to the evaluation of the efficacy of the diet and lifestyle modification intervention. The Electrical Impedance Mini-Lab will permit the determination of serial quantitative body composition measurements via bioelectrical impedance. The equipment will be used in the initial assessment and subsequent intervention and evaluation phases of the program. In addition to providing baseline and post-intervention data, body composition data will serve as a motivator providing feedback to participants in the intervention phase of the program. Improvements in health and nutritional status, in this high risk group, and the enhancement of their ability to make health-promoting food choices is of significant benefit both to U.S. agriculture and society.

9901472 Practical Production of Bacterial Cellulose
Bungay, H.R.
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Strengthening Award; Grant 99-35504-8011; $70,000; 1 Year

Cohesive cellulose films are formed by *Acetobacter xylinum* in a rotating disk bioreactor with relatively dilute solutions of sugars. The product is pure cellulose as a highly hydrated gel from which the organisms are removed easily. Much of the water can be squeezed out leaving cellulose to dry quickly to a very tough sheet for such applications as making acoustic diaphragms for headphones and loud speakers. The disk reactor has a high surface area for film growth, and a run takes 3 to 4 days in contrast to the old surface culture method that takes 7 to 14 days. Different solid materials can be incorporated into the gel to make gradients of solids, stripes of solids, and bands. There are existing and potential applications for both the wet gels and dry sheets of cellulose and its composites with solids for medicine, electronics, food, and paper. This project will address adapting this system to agricultural practices in the United States and testing more solids that will inspire new applications. Specific goals are obtaining data on which scale up can be based, improving methods for harvesting the product from the disks, defining mixing conditions for good incorporation of solids into the gel as it forms, characterizing properties of gels and dried sheets that are composites of cellulose and various solid particles, surveying new applications, and analyzing costs of the proposed processes.

9901125 Hydrologic Control of Nitrogen Transport at Hydrogeomorphic Interfaces in a Forested Watershed
Cirmo, C.P.
SUNY College, Cortland; Department of Geology; Cortland, NY 13045
Strengthening Award; Grant 99-35102-8588; $100,000; 2 Years

Recent evidence indicates the importance of hydrogeomorphic interfaces (e.g., bedrock/till, till/peat, peat/alluvium, alluvium/channel) in controlling temporal N transport in streams. Underlying glacial deposits and deep groundwater reservoirs may be a major contributor to N pulses seen in many eastern streams, but locating and sampling them are problematic in these glacially-mantled watersheds. We are using spatial and temporal sampling strategies to characterize the contribution of these deep sources of N to a stream channel on a continuum from hillslope, wetland, hyporheic and littoral zone waters in an Adirondack watershed in New York. Ground penetrating radar (GPR) will assist us in locating the depth of deep groundwater sources, in addition to the vertical heterogeneity in hydraulic conductivity in wetland and hyporheic deposits. Measurements of water table fluctuations within wetlands intersecting primary drainage channels (hydroperiod determination), along with tracing of preferential flow controlled by alternating layers of peat and alluvium, will assist in the determination of the spatial and temporal contribution of deep N sources to streamwater N-concentrations. In addition, subchannel sampling of hyporheic inputs (mini-piezometers) will allow us to interpret the seasonal and episodic contribution of hyporheic deposits to N-signature. Results will be generalized to the region through ground-truthing at other wetlands within the Upper Hudson River basin. This information will be coupled to a digital elevation model (DEM) of the watershed, and long-term monitoring of overall N flux from the watershed, to develop a better understanding of N pollution inputs, and to determine the most critical hydrogeomorphic interfaces controlling N transport.

9903587 Cloning of Guard Cell Specific Genes by Enhancer Trap Tagging

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Seed Grant; Grant 99-35106-8245; $50,000; 2 Years

Two of the most important interactions between a plant leaf and its environment are the uptake of CO_2 and the loss of water vapor from the leaf by transpiration. The exchange of these two gases occurs simultaneously through pores in the surface of the leaf, which are formed by pairs of kidney-shaped cells in the epidermis, called guard cells. Guard cells can open the pore, to allow maximum uptake of CO_2 for photosynthesis, but then close it under times of drought to reduce water loss by transpiration. Greater understanding of the regulation of guard cell function may provide us with the ability to generate plants with enhanced drought tolerance. This research is aimed at identifying proteins likely to be involved in guard cell structure and/or function because the gene encoding that protein is only activated in guard cells. We will screen through thousands of Arabidopsis plants transformed with a gene (a reporter gene) that results in blue staining only in the cells where that gene is activated. In each of these thousands of plants, this reporter gene has inserted into a random spot on a chromosome, and in rare occasions it will have inserted into a gene that is only activated in guard cells. We will identify those few plants that display blue staining only in guard cells, then use molecular cloning techniques to isolate and characterize those genes that have been tagged by the reporter gene. Cloning of one such tagged gene is underway.

9903582 Insecticidal Petuniasteroids and their Analogs: Synthesis and Bioactivity

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Seed Grant; Grant 99-35311-8272; $50,000; 2 Years

Petuniasteroids are a group of natural insecticides originally isolated from petunias at the Western Regional USDA Center. These substances exhibit potent insecticidal activity, but research has been hampered by the small quantities available from petunia plants. The chemical synthesis of petuniasterone D has recently been accomplished in the P.I.'s laboratory. This synthetic procedure will be the starting point for the synthesis of analogs of the petuniasteroid molecule designed to assess the minimum structural parameters essential for insecticidal potency. The insect bioassays will be carried out in collaboration with insect toxicologists and will build on previous studies using the corn earworm (*Heliothis zea*) as the primary test organism. The substances prepared in the course of this research will be made available to insect and mammalian toxicologists for collaborative studies on their mechanism of action.

9901756 Quinone Dependent Electron and Proton Transfers in Bacterial Reaction Centers

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Strengthening Award; Grant 99-35306-7792; $120,000; 2 Years

Plants and photosynthetic bacteria have similar proteins that efficiently transforms light into chemical energy. The bacterial photosynthetic reaction centers (RCs) are smaller than those from plants and have a known 3-dimensional structure. The proposed studies focus on the RC quinones. Many commercial herbicides bind to PSII at a site homologous to the one that will be studied here. RCs bind quinones in 2 locations. An electron is transferred from the primary (Q_A) to the secondary (Q_B) quinone in the last of the intra-RC electron transfers that are initiated by light. Protons are bound to the protein on this reaction adding to the stored energy. For the reaction from Q_A to Q_B to be favorable, the protein must change the chemistry of each quinone. Studies will highlight the role of the protein in determining the quinone chemistry and in controlling the electron transfer and proton uptake. (1) The electron transfer from Q_A to Q_B is limited by some change in the

protein. RCs have recently been prepared where the reaction can go much faster because the conformational change is not needed. The fast reaction will be characterized and the reasons for the conformational change determined. (2) The protein must modify the quinone chemistry so the electron transfer is favorable. Different compounds will be tested in the Q_B site to see how the protein changes the properties of bound quinones. (3) The changes of the protein structure that are required for the reaction will also be studied by measurements at very low temperatures.

9903653 A Calorimetry System for Study of Small Ruminant Pastoral Energetics
Sahlu, T.
Langston University; E (Kika) de la Garza Institute for Goat Research; Langston, OK 73050
Equipment Grant; Grant 99-35208-8538; $50,000; 2 Years

Forages provide most nutrients to ruminants throughout the world and, in most instances, are consumed through grazing. However, the great majority of nutrition research with ruminants has been in confined settings. In order to design optimal grazing management strategies, encompassing considerations such as forage quality and available forage mass, it is necessary to thoroughly understand nutrient costs of grazing. To gain such knowledge, a calorimetry system to measure heat production is necessary to establish relationships in different experimental settings between indirect measures during grazing and heat production or energy use. Thus, a calorimetry system would greatly enhance the ability of the E(Kika) de la Garza Institute for Goat Research of Langston University to accurately estimate effects of grazing conditions on energy expenditure by small ruminants. Objectives of this project are to install a four-animal calorimetry system and determine energy expenditure by goats grazing warm or cool season grasses at different available forage mass levels by the indirect carbon dioxide entry rate technique used while grazing. After completion of this project, a series of experiments will be devised and conducted to elucidate effects of a wide array of grazing management systems and treatments on energy loss due to grazing and, thus, energy accretion, secretion in milk, and(or) use in tissue maintenance.

9903673 Fluorescence Sequence Detection System to Enhance Animal Health Research
Malayer, J.R.
Oklahoma State University; Department of Infectious Diseases and Physiology; Stillwater, OK 74078-2006
Equipment Grant; Grant 99-35208-8403; $47,500; 1 Year

The purpose of this project is the acquisition of a system for detecting specific DNA sequences using fluorescent-tagged probes. The project will use matching funds provided by the Oklahoma State Regents for Higher Education to purchase the ABI Prism 7700 Sequence Detection System. Multiple investigators in the Colleges of Veterinary Medicine and Agriculture at Oklahoma State University will make use of this instrument. Applications of this technology include molecular genetics, gene expression, immunology, and infectious disease research. There will be significant enhancement of ongoing research in genetic analysis of disease-causing viral agents, genetic analysis of animal host immune responses to disease pathogens, as well as genetic analysis of the healthy animal. The instrument provides significant improvements to technology currently in use. Many of our studies require amplification of specific DNA sequences from very small amounts of starting material by a method called polymerase chain reaction. Variation in the polymerase chain reaction technique often results in loss of the quantitative differences present in the starting material, thus important information may be lost as a result of the need to amplify the DNA sequence. The new system operates by detecting the increase in a fluorescent probe signal released with each cycle of amplification of the specific target DNA sequence. Because of the capability to measure and record this variation in the polymerase chain reaction amplification, the new technology allows for reliable measurement of small amounts of specific DNA sequences with far greater accuracy than was achievable by previous methods.

9903712 Acquisition of a Fluorescence Microscope
Ding, B.
Oklahoma State University; Department of Botany; Stillwater, OK 74078-3013
Equipment Grant; Grant 99-35311-8650; $7,152; 1 Year

This project is to purchase a Nikon epifluorescence microscope to meet the increasing needs of research activities in my laboratory. Research in my lab is currently being supported by two USDA NRICGP grants (one from the Plant Growth and Development program and the other from the Plant Pathology program) and two Samuel Roberts Noble Foundation grants. All research activities are in the areas of intracellular and intercellular transport of proteins and nucleic acids in relation to viral movement in their hosts and plant growth and developmental regulation. These activities involve extensive use of fluorescence microscopy in addition to other routine microscopic methods. The current Nikon Optiphot-2 light microscope in my laboratory can hardly meet the increasing demands of my research group. The addition of a second fluorescence microscope will allow us to maintain current research activities without losing productivity as well as to enhance our capability to continue exploring new frontiers in plant biology. Overall, the requested microscope will facilitate greatly our research in understanding how viruses move systemically in a host plant and how cells communicate with each

other during plant growth and development. The knowledge gained from such research could be useful in designing crops for improved resistance against pathogens and for improved agricultural value.

9903428 Development and Assessment of an Optimized Lure and Trap System for Female Moths

Reed, H.C.
Oral Roberts University; Biology Department; Tulsa, OK 74171

Sabbatical Grant; Grant 99-35311-8530; $57,105; 1 Year

This research proposes to investigate the use of feeding attractants *for female* pest moths in integrated pest management (IPM) programs. Traditionally, most attractants used in IPM programs are used to lure males of the species, which do little damage to the crop plant. Consequently, a trapping scheme using attractants for females, who produce the larvae that destroy crop plants, would be more effective at reducing pest populations and reduce crop damage. The use of these attractants in a trapping scheme may reduce the use of pesticides as it would improve the timing and minimize the number of pesticide applications in the field. The primary objective of the proposed research is to develop an optimized lure and trap system *for females* of two species, the corn earworm, *Helicoverpa zea* and the spotted cutworm, X*estia c-nigrum;* species which feed and harm several crop plants. In addition, the project seeks to determine if such a sampling system *for females* will be a more accurate measure of the egg-laying and development of these pest moths in the field than the current imprecise method of using traps baited with attractants to lure males only. The optimization of the lure and trap will be conducted in commercial and experimental plantings of corn and cabbage. Initially a range of lure release rates will be tested in order to determine the best release rate and then subsequently the optimal dose will be tested in 6 different trap designs. The female-based moth sampling method will be compared to the male sampling method to determine if a female moth lure and trap is the more reliable estimate of the developing pest population and thus, a more effective IPM tool.

9903588 Production of Antibodies to *Zea mays* Sperm

Southworth, D.
Southern Oregon University; Department of Biology; Ashland, OR 97520

Seed Grant; Grant 99-35311-8653; $50,000; 2 Years

The long-term goals of this Seed Grant are to discover the molecular events of fertilization in flowering plants, specifically, to determine which molecules on sperm cell surfaces participate in events of recognition, adhesion, and fusion with the egg. From this research, eventually we will be able to identify the genes responsible for fertilization and then to engineer them in such a way as to promote or prohibit fertilization—to regulate fertility and sterility in plants. Plant sperm are embedded in pollen grains and move down growing pollen tubes. We will collect corn pollen and isolate plant sperm cells from it by gentle grinding. The sperm will be purified and used to immunize mice. Undergraduate students will screen the antibodies produced to determine which ones bind to sperm cell surfaces. Second, we will determine whether any of the antibodies that recognize sperm cell surfaces bind to only half the sperm and thus may be membrane components potentially responsible for double fertilization in flowering plants. Third, we will determine the molecular weight of the antigenic molecules from corn sperm cells. Undergraduate students will gain valuable insight into active research. By solving problems and assisting in attainment of project goals, they will gain experience in understanding the approach to research and in development of technical skills which will facilitate their development as scientists.

9903455 Scallop Restoration Study: Ultra-Cold (-80) Freezer

Scott, T.M.
Roger Williams University; Department of Biology; Bristol, RI 02809

Equipment Grant; Grant 99-35208-8518; $15,795; 2 Years

This grant provides funds for the purchase of an ultra-low temperature freezer that will support the aquaculture research program at Roger Williams University. These freezers hold biological samples at temperatures down to -86°C making them ideal for the long-term storage of tissue samples, plasma, enzymes and molecular transcripts. To date, the lack of this low temperature capability has seriously limited our ability to apply molecular and genetic techniques to aquaculture research. Initial plans for the freezer include storing samples and assay chemicals for a genetic study of scallop restoration efforts in the Westport River. Historically, this estuary supported a large, profitable harvest of the bay scallop *(Argopecten irradians),* however this declined by the late 1980's as a consequence of over-harvesting and pollution. A recent increase is credited to a concerted, grass-roots restocking effort in which juvenile scallops are collected each Spring, held until large enough to avoid predation and then released. Given the inherent variability of shellfish recruitment, it's uncertain whether restocking or natural factors are responsible for the increase. We will use protein electrophoresis, a basic technique in population genetics, to address this question by looking for genetic differences between the released scallops and those found naturally in the estuary. It's anticipated that the results will have general applicability to shellfish restocking efforts elsewhere.

9902902 Optical Biosensor Detection of Food Pathogens Based On Direct Measurement of Antibody/Antigen Binding

Rand, A.G.; Pivarnik, P.; Letcher, S.; Brown, C.W.; Goldberg, B.B.
University of Rhode Island; Food Science and Nutrition Department; Kingston, RI 02881
Strengthening Award; Grant 99-35201-8435; $180,000; 2 Years

The demands from consumers for fresh, less processed food has increased the need to ensure microbial safety of these products. This project provides the opportunity for a multi-disciplinary effort to create specific biosensors for rapid and early detection of pathogen contamination. These devices have the potential for specifically selecting food pathogens from among the total microbial load within minutes and measuring the concentration as real-time analysis on site. The goal of this project is to develop new prototype biosensor devices that will consist of either a fiber optic fluorometer and magnetic focusing system or a new evanescent thin-film wave guide for the transducers. One advance will be the creation of an immunosensing element which consists of unique new complexes of antibodies attached to solvatochromic fluorescent dyes. This new dye/antibody complex will be designed to detect cell attachment directly when immobilized on magnetic microspheres or thin-film wave guides. The coupling of direct measurement antibodies with laser excitation will provide the basis for rapid one-step, sensitive, and easy-to-use biosensors for detection of food pathogens. The ability for rapid early detection of *Salmonella* will enhance the safety and quality of U.S. food products.

9901358 Intralocus and Intragene Recombination in the *Gli-B1* and *Glu-B3* Loci in Wheat
Lukaszewski, A.J.; Clegg, M.T.
University of California, Riverside; Department of Botany and Plant Sciences; Riverside, CA 92521
Strengthening Award; Grant 99-35301-7593; $120,000; 2 Years

We will study the pattern of recombination in the multigene loci *Gli-B1* and *Glu-B3* in wheat, encoding gliadins and low molecular weight (LMW) glutenins. These two storage proteins contribute over one half of the observable variation in the technological properties of wheat. Because of very tight linkage between the two, little is known about the effects of allelic variation at each locus, their organization, or the contribution of individual genes to the properties of wheat. We will saturate the critical region of the chromosome arm lBS with at least 800 cross-overs per 1 cM produced at two levels of chromosome pairing stringency control (normal, *Phl*, and mutant, *phlb)* by using a recombination-enhancing translocation and screening for flanking markers. This should let us determine the number and the order of genes present at each locus, to break linkages between the two loci and between individual genes in each locus, and to generate new hybrid alleles. We will develop PCR methods for high resolution mapping within the loci and to produce DNA markers for individual alleles. We expect that the materials and methods developed in this study will let us define in the future the criteria of the DNA sequence similarity for crossing over as well as generate convenient DNA markers for various alleles of storage proteins for plant breeding. This study will also generate genetic stocks of wheat for field tests of the technological properties of new genetic variation.

9903708 Image Analysis in the College of the Environment and Life Science
Gorres, J.H.; Amador, J.A.; Husband, T.P.
University of Rhode Island; Department of Natural Resources Science; Kingston, Rl 02881
Equipment Grant; Grant 99-35106-8243; $50,000; 1 Year

Research in the College of the Environment and Life Science includes the analysis of image data from a wide range of projects. A flexible image analysis system will increase the accuracy and speed of these analysis. Soil pore structure regulates the consumption of microorganisms by nematodes and protozoa, an interaction that enhances the recycling of nutrients and thus their availability to crops in soil ecosystems. Pore structure also determines in part the accessibility of soil pollutants to microorganisms that would degrade them. Pore structure measurements with image analysis will improve our understanding of the ecology of nutrient supply in agro-ecosystems, big-degradation of pesticides and organic matter in shallow narrow field septic systems. These projects aim at improving the efficiency of US agriculture and the quality of rural life. The diversity of soil organisms is an important measure of soil health. Genetic fingerprinting of soil communities may provide indicators of diversity. Image analysis will be used to measure images of genetic data. Greater use of mechanical pulps would improve fiber utilization efficiency. However, mechanical pulp structure and properties are often inferior to those of chemical pulps. The measurement of fiber properties with image analysis will provide data to formulate improved and competitive mechanical pulp blends. The control of plant parasitic nematodes is essential in many cropping systems. Alternative pest management strategies to toxic nematicides are being developed. Understanding the structural and physiological damage to nematodes caused by the alternative pest control agents would help in improving the efficiency of these methods.

9903656 Impedance Analyzer for Piezoelectric Biosensor Research
Letcher, S.; Rand, A.G.
University of Rhode Island; Department of Physics; Kingston, RI 02881
Equipment Grant; Grant 99-35207-8541; $13,832; 1 Year

Foodborne illnesses exact a heavy toll in human suffering and lost production. Since foodborne pathogens are difficult to eliminate entirely, the best defense begins with rapid detection. This project expands and refines the study of biosensors for the efficient and rapid detection of pathogens. A vibrating piezoelectric crystal will detect the mass of captured pathogens and their viscoelastic properties. The additional information gained about the capture process will lead to improved biosensors for food safety. The proof-of-concept phase will concentrate on the detection of Salmonella, but the system can easily be adapted for the detection of *E.Coli*, vibrios, and other dangerous pathogens.

9903266 Improving Flounder Productivity through Enhancing Growth

Specker, J.L.; Bengtson, D.A.
University of Rhode Island; Graduate School of Oceanography; Narragansett, RI 02882-1197
Strengthening Award; Grant 99-35206-8585; $119,000; 3 Years

The United States imports $7 billion/year in excess of its seafood exports. In Europe and Asia, land-based flatfish farming has been a tremendous success and the immediate market for summer flounder in the United States is estimated at $20-30 million/annually. This year, for the first time, land-raised summer flounder were brought to market in the United States. A problem faced by growers is the variability in early growth rates. Because female flatfish out grow males by 2- to 4-fold, we propose to produce all-female crops of summer flounder. This is unusual because the effort in catfish and trout farming has been to produce male crops, which would support greater productivity. The likelihood of our success using estradiol treatment during a critical stage of gonad differentiation is high, based on parallel studies in model fish species. Rapid application of the assistance we propose would improve productivity and the cost:benefit ratio greatly, especially at this early stage in the industry's development.

9903735 The Nutritional Ecology of Birds in Relation to Environmental Change

McWilliams, S.R.
University of Rhode Island; Department of Natural Resources Science; Kingston, Rl 02881
Equipment Grant; Grant 99-35106-8241; $23,820; 1 Year

The proposed research evaluates how birds that inhabit coastal, managed forests in southern New England during their migration respond to natural and human-induced environmental change. At stopover sites in these coastal forests, songbirds interrupt their migratory journey between breeding and wintering areas to rest and refuel. Despite the obvious importance of stopover sites for recovery of stored energy and nutrients, the ecology and physiology of birds at stopover sites during migration is considered the least studied topic in bird migration. The nutritional status of an animal depends on its nutrient needs and the availability and accessibility of nutrients in the environment. Determining the adequacy of certain environments for animals would be relatively simple except animals exercise plasticity in behavior, morphology, physiology, and metabolism. Our study of small passerine birds during migration focuses on an ecologically important form of organismal plasticity: the dynamics of body composition and digestive organs and its nutritional significance. We propose to use blood metabolites and measures of body composition to assess nutrient utilization in small songbirds. Our studies of nutrient transport and hydrolytic activity of digestive enzymes in birds allows critical points in nutrient absorption and metabolism to be identified during productive (i.e., nutrient storage) periods such as migration. Evaluating how migratory birds respond to environmental change and defining the limits of their response(s) during migration allows more reliable and accurate predictions about the effects of environmental change on the sustainability of forest ecosystems under certain management practices.

9903710 Image Acquisition and Analysis: An Equipment Grant Proposal

Marcotte, Jr., W.R.
Clemson University; Department of Biological Sciences; Clemson, SC 29634
Equipment Grant; Grant 99-35311-8251; $35,000; 1 Year

In molecular biology, genetics and biochemistry research, biological molecules like DNA, RNA and protein are routinely monitored, isolated and characterized. These molecules are involved in all cellular processes and provide vital information about everything from normal development and cellular housekeeping functions to disease states and responses to biotic and abiotic stimuli. They are frequently the most sensitive indicators of the physiological status of agriculturally important organisms, both plant and animal. However, detection and/or visualization of biomolecules is indirect through radioactive, fluorescent, chemiluminescent, chemifluorescent or visible stain labeling. Therefore, it is imperative to have a sensitive, and frequently quantitative, method with which to study these molecules. It is also essential for documentation of results from research projects for use in scientific publications, in grant proposals and for general laboratory record-keeping.

This award will allow the purchase of an image acquisition and analysis system for use by several laboratories. The instrument sought will allow high resolution detection and/or visualization (over ~5 orders of magnitude sensitivity), quantification and analysis of biomolecules through the use of fluorescent and chemiluminescent/chemifluorescent technologies. Some of the specific enhancements will include the ability to accurately monitor and quantify protein expression, to detect and quantify specific messenger RNA (mRNA) levels, to analyze mRNA populations and to fingerprint

genomic DNA clones for mapping and positional cloning. Further, the addition of a state-of-the-art image acquisition and analysis system will allow us to move into new areas of research through the application of new and emerging detection technologies.

9902450 Load-Sharing and System Factors for Light-Frame Walls

Rosowsky, D.V.
Clemson University; Department of Civil Engineering; Clemson, SC 29634-0911

Strengthening Award; Grant 99-35103-8543; $120,000; 2 Years

The wood industry is facing unprecedented competition from other structural materials in the light-frame construction market in the United States. Clearly the primary framing material is dimension lumber, and while advances in the production and use of engineered wood composites is certain to continue, conventional construction using dimension lumber will remain the most widely used for light-frame construction. One promising mechanism for increasing the competitiveness of wood as a structural material, and more specifically, as a material for engineered construction, is the use of repetitive-use (or system) factors in design. These factors take into account the fact that individual structural members are actually performing as part of a structural system, with inherent load-sharing. The proposed research seeks to address the need for improved design procedures that account, in a rational and defensible way, for load-sharing and load-distribution effects in conventional light-frame walls. This, in turn, will serve to increase the competitiveness of wood as a structural material and ensure its continued use in the light-frame market.

993557 Chromosome Mapping in Rose and Its Application to Marker Assisted Selection

Ballard, R.
Clemson University; Department of Biological Sciences; Clemson, SC 29634-1902

Seed Grant; Grant 99-35311-8374; $49,990; 2 Years

Roses are among the most important ornamental crops worldwide. In the US alone, garden roses account for $300 million in annual sales to 23 million households. There are approximately 20,000 commercial rose cultivars, most of which are tetraploid (x=7) and have been derived from eight to 10 wild diploid and a few tetraploid rose species. Our long term goal is to construct a DNA marker based genetic linkage map for rose, to understand the genetic basis of important floral and other traits in roses, and to identify markers linked to these genes. Specific traits that will be studied under the scope of this proposal are flower color, petal number, absence of prickles on stem and petiole, bush form and resistance to black spot. Genetic markers identified as tightly linked to these traits will be used in a marker-assisted selection scheme to identify breeding lines and track introgression of the economically important traits (e.g., thornless stems, disease resistance) into genetic backgrounds of modern hybrid Tea roses. The objectives of this project include the following: (1) to saturate the current linkage map to obtain markers with an average spacing of ScM, (2) to understand the inheritance and genetic basis of the traits, (3) to locate these genes on the rose map developed, and (4) to identify those markers tightly linked to traits by bulked segregant analysis.

9903507 Peri-implantation Platelet-activating Factor Receptor Expression in Pig Embryos and Endometrium

Diehl, J.; Roudebush, W.E.
Clemson University; Department of Animal and Veterinary Sciences; Clemson, SC 29634

Seed Grant; Grant 99-35208-8163; $50,000; 2 Years

The primary project goal is to expand our knowledge on the role of platelet-activating factor (PAF) in pig reproduction with a goal of increasing reproductive efficiency and improving reproductive management. PAF is present in pig uterine fluid. Preimplantation embryos produce and secrete PAF. The amount of embryo-derived PAF produced is correlated to pregnancy potential. PAF plays a significant role in preimplantation embryo development, being required for growth and implantation. Embryos cultured with added PAF have enhanced growth and higher implantation rates. Moreover, PAF antagonists inhibit development and implantation indicating the presence of a PAF specific receptor. PAF's signal mechanism, in most cell types, is receptor mediated, but only recently has PAF function been characterized with few reports on PAF's presence in livestock species. We hypothesize that PAF-receptors are present in pig embryos and the uterus and that their levels are highest at the time of implantation. Therefore, the objective addressed in this proposal will verify and quantify the presence of PAF-receptors in pig embryos and uterus around the time of implantation.

9903718 Genetic Analysis for Multi-User Analytical Laboratory

Riley, M.B.
Clemson University; Department of Plant Pathology and Physiology; Clemson, SC 29634

Equipment Grant; Grant 99-35106-8123; $38,925; 1 Year

The identification of bacterial organisms is important to many research areas within the University including biotechnology, environmental and toxic water management, water quality analysis, and food quality and safety. During the past nine years bacterial identification has been conducted using gas chromatographic analysis of fatty acids extracted from the bacteria. This system has been widely used by a large number of research groups and teaching laboratories on campus. However, analysis of fatty acids for bacterial identification requires specific growth conditions since changes in growth media, temperature, and time of growth in culture can affect both the quantity and quality of fatty acids resulting in problems matching the fatty acid profiles in the commercial and user generated library entries. The addition of a DNA genetic analyzer and associated software to the Multi-User Analytical Laboratory for the identification of bacteria based on ribosome gene sequence data would greatly enhance and expand the research and teaching capabilities at Clemson University. Ribosomal genes are involved in the production of ribosomes crucial to the production of proteins in the cell and their sequence has been shown to be a good tool in the identification of bacterial organisms. Sequencing of the ribosomal gene overcomes the problems associated with the use of fatty acid production for bacterial identification. The addition of a ribosomal gene analysis system would improve our ability to identify organisms and make comparisons of various microorganisms by complementing the fatty acid analysis system currently available.

9902281 Origin and Significance of Turkey Seminal Plasma Proteolytic Enzyme(s)

Thurston, R.J.
Clemson University; Department of Animal and Veterinary Sciences; Clemson, SC 29634-0361

Strengthening Award; Grant 99-35203-8161; $120,000; 2 Years

The domestic turkey is unique among avian species in that a proteolytic enzyme (TSPE), active at basic pH, is found in seminal plasma. The objectives of this research are to: 1)determine the origin of TSPE within the reproductive tract; 2)biochemically characterize TSPE; and 3)investigate the effects of TSPE or TSPE inhibitors on sperm mobility and fertilizing capacity of stored semen. A monoclonal antibody to TSPE will be produced and used to immunostain sections of the turkey male reproductive tract to determine sites of enzyme production and/or concentration. Enzyme kinetics will be studied using synthetic substrates and proteinase inhibitors. TSPE activity will be determined in pooled turkey semen immediately after collection and periodically during storage at 4C for up to 48 hours. The effects of adding TSPE or proteinase inhibitors to semen during storage will be determined by evaluating sperm mobility. If addition of TSPE or proteinase inhibitors to stored semen results in improved sperm mobility, fertility trials will be conducted to determine if a similar beneficial effect is observed for sperm fertilizing capacity from semen stored for 24 hours. Fertility, hatchability of eggs, and embryonic mortality will be examined. The knowledge generated from this research will add to the understanding of avian semen physiology and may result in improved methods for semen storage. This would benefit the turkey breeding industry, where semen is routinely pooled and stored prior to use for artificial insemination.

9903531 Isolation of Defined Genome Regions via Homologous Recombination

Frisch, D.
Clemson University; Clemson University, Genomics Institute; Clemson, SC 29634

Seed Grant; Grant 99-35311-8408; $49,978; 2 Years

The discovery, isolation and characterization of genes are important goals of plant scientists. The study of isolated genes increases our knowledge of how plants function and leads to new ideas for improvements in crops to help feed an expanding world population. One popular method for gene isolation is called chromosome walking. This method makes use of genetic markers closely linked to a gene or trait of interest. The markers are used ro isolate genomic clones containing the markers. The genomic clones are then characterized and used to isolate new set of clones that overlap. This cyclical cycle, or walking, is continued until the region of interest has been isolated. The rate of progress depends on the size of clones, or steps. that can be isolated and the entire process can be very time consuming and expensive. The method proposed here has the potential for the direct isolation of large (>200 kb) genomic regions using the active recombination system present in yeast. By cloning target DNA molecules into a vector and then transforming the linearized vector and genomic DNA into yeast, the intervening sequence can be rescued as a circular plasmid via recombination between the homologous sequence in the target DNA and the genomic DNA. The plasmid can then be isolated from yeast and used to transform bacteria where many to the molecular biology techniques are easier. This technology could provide a major advancement in the isolation and comparison of genomic regions between inbred lines or between cultivated crops and wild related species.

9903614 Bovine Relaxin-like Factor (bRLF) and its Function

Bullesbach, E.E.
Medical University of South Carolina; Department of Biochemistry; Charleston, SC 29425
Seed Grant; Grant 99-35208-8535; $50,000; 2 Years

The relaxin-like factor (RLF) was thought to be specifically expressed in testicular Leydig cells. Knockout mice resulted in infertile males but had little effect on females. The chemical synthesis of RLF and the subsequent generation of antibodies revealed that the relaxin-like factor is a circulating hormone in humans and has specific receptors on mouse uteri and brain. In both species the level of expression in female reproductive tissues is distinct but very low.

The situation is different in ruminants where high levels of RLF expression was found in testicular Leydig cells and in the ovaries. In cows RLF expression is cycle dependent and is high during pregnancy. It has been hypothesized that RLF affects sperm maturation in males, augments relaxin activity in females and might even substitute for relaxin in cows. Consequently, in bulls RLF has the potential to affect sperm counts and in cows it might support the widening of the birth canal and softening of the cervix to improve calving.

The goal of the present study is to use synthetic bovine RLF to develop a specific immunoassay system. This assay will be utilized to: 1) determine whether or not bovine RLF is a circulating hormone; 2) obtain some biochemical characteristics of native bovine RLF; and 3) quantify the hormone in serum of sexually mature and immature males and females. In the long-term RLF levels will be correlated with the appearance of other sex hormones and eventually correlated with reproductive success.

9902315 Disease Resistance Lowered by Dissolved Oxygen and pH in Aquacultured Shrimp

Burnett, L.E.; Burnett, K.G.
University of Charleston, SC; Grice Marine Laboratory; Charleston, SC 29412
Strengthening Grant; Grant 99-35204-8555; $155,000; 2 Years

Dissolved oxygen has been widely used as a measure of water quality and has been useful in protecting animals and their habitats. The deleterious effects of acute exposure to low oxygen (hypoxia) on shrimp survival are well known. However, little is known of how chronic or periodic hypoxia and elevated water CO_2 (hypercapnia; a concomitant with low oxygen) alter the resistance of juvenile and post-larval shrimp to pathogenic infections of bacteria, viruses, and fungi. In commercial production, shrimp are stocked at high densities and frequently subjected to low oxygen stress. Preliminary data suggest that hypoxia greatly reduces the resistance of shrimp to bacterial infections. We will use an experimental model to assess the influence of hypoxia on the ability of juvenile and post-larval shrimp to resist infection with a common opportunistic pathogen under typical aquaculture situations. We will identify the duration and the intensity of low oxygen that places production stocks at risk for massive losses from bacterial disease. The shrimp, *Penaeus vannamei*, held at non-lethal levels of hypoxia will be exposed to the bacterial pathogen, *Vibrio parahaemolyticus*, and their survival quantified. The results of these studies will establish oxygen levels critical to maintaining resistance against opportunistic infections in shrimp.

9903602 Health and Management Factors Affecting Beef Value

Fausti, S.W.; Johnson, B.J.; Epperson, B.W.
South Dakota State University; Department of Economics; Brookings, SD 57007-0895
Seed Grant; Grant 99-35402-8657; $50,000; 2 Years

The US beef industry is segmented such that numerous individual producers may be involved managing the production of any single animal. This structure has the positive connotation of "traditional" agriculture. However, segmentation has resulted in a decrease in beef industry competitiveness.

Our goal is to develop a data set which will allow us to identify health and management factors throughout the beef production chain that contribute to feedlot performance and beef value. While we are not the first to approach this goal, our methodologies are unique in that they focus on describing efficiency as a function of feedlot and ranch management, utilizing a "life cycle" problem solving approach.

As our initial objective, we will construct an electronic database and perform specific analyses of factors affecting feedlot performance, carcass quality and tenderness, total calf value, and presence of lung lesions at processing. These outcome variables were chosen because they are important determinants of economic value and are areas of emerging interest. We will use a unique study population that will minimize the effect of "feedlot" but maximize the range of explanatory variables.

This project is linked to major industry concerns. It uses both biologically and economically derived value outcomes and relates these to disease prevention/management variables at play in the beef production cycle. The data generated as a result of this study can be used to assist producers to improve efficiency through elucidation of management techniques that optimize food production.

9903603 Corn-Based Production of Commercially-Available Gellan Gum

West, T.P.
South Dakota State University; Olson Biochemistry Laboratories; Brookings, SD 57007
Seed Grant; Grant 99-35501-8419; $50,000; 2 Years

The objective of this project is to develop a process for the corn-based production of the commercially-available bacterial gum gellan. This research will involve the isolation of antibiotic-resistant mutants of the bacterium *Sphingomonas paucimobilis* and the subsequent screening of the isolated antibiotic-resistant mutants for an increased ability to elaborate corn-based gellan relative to the ability of their parent strain. Once such mutants exhibiting elevated gellan gum from corn syrup have been identified, it will be necessary to study the gellan gum produced by these mutants to ensure that they are truly synthesizing authentic gellan. This will involve the use of various sensitive and analytical biochemical separation techniques to scrutinize the structure of the gum produced and compare it with the known structure of commercially-produced gellan gum to confirm that the gum is authentic. After showing that the gum produced by the mutants is authentic, it should be possible to use these strains in the large-scale commercial production-of corn-based gellan gum. The importance of this research to agriculture is that the production of a corn-based gum represents a new non-food use for corn and a value-added product. With gellan gum being commercially-available and with the constantly increasing annual demand for bacterial gums, a corn-based gellan gum could greatly increase corn utilization. This should not only elevate the price of a bushel of corn but also reduce the importation of gums from foreign markets into the United States.

9903430 Molecular and Genetic Mechanisms Involved in Bud Dormancy in Woody Plants
Fennell, A.
South Dakota State University; Horticulture, Forestry, Landscape and Parks Department; Brookings, SD 57007
Sabbatical Grant; Grant 99-35311-8324; $63,606; 1 Year

Temperate-zone woody plants undergo dormancy which is a phase of development that contributes to the ability of woody plants to survive unfavorable winter conditions. The timing of dormancy induction and release is an important factor in economic production of deciduous fruit crops, ornamental and forestry trees and shrubs. While extensive studies have been made in the physiological aspects of the bud dormancy cycle, molecular techniques provide the potential to identify regulatory pathways and mechanisms responsible for dormancy induction and release in woody plant buds. The objectives of this proposal are to identify and characterize differential gene expression and genes that are associated with dormancy in woody plants.

The proposed research will be conducted in cooperation with a scientist at Oregon State University. It will use two different woody plant genetic systems, grapes and poplar, that show differential response for photoperiodic induction of dormancy. Two molecular expression techniques will be used with RNA isolated from the responsive and nonresponsive plant materials to identify and isolate dormancy specific markers. Cross characterization of the differentially expressed markers in these two different species will help identify factors that are common between species and factors that are species specific during induction of dormancy. The information and molecular markers developed from this research can provide tools to study the reception of environmental signals and transduction to dormancy induction and acclimation processes in woody plants. The studies have the potential to provide tools for breeders and geneticist to use in marker assisted breeding and selection programs.

9903675 Acquisition of an ABI PRISM 310 Genetic Analyzer
Rowland, R.R.; Chase, C.C.; Duvall, M.R.
South Dakota State University, Department of Biology-Microbiology; Brookings, SD 57007
Equipment Grant; Grant 99-35208-8420; $33,550; 1 Year

Improvements in agriculture are becoming more dependent on understanding the function of plant, animal, and microbial genes. The principal tool in gene analysis is the deciphering of DNA sequence information. The estimated 1,000 samples subjected to DNA sequencing within South Dakota State University's College of Agriculture and Biological Sciences last year illustrate the growing dependency of research on DNA sequence information. The purpose of this proposal is the acquisition of an automated DNA sequencing system, ABI PRISM 310 Genetic Analyzer. This system will support research in the areas of biotechnology and infectious disease. Specifically, this system will be incorporated into 14 projects within the College of Agriculture and Biological Sciences, which includes research on porcine reproductive and respiratory virus, enterotoxigenic *E. coli*, cattle viral diseases and genetically improved soybeans, cattle, pigs, and wheat.

9903646 Gas Chromatograph Mass Spectrometry of Oilseeds Value-Added Processing and Characterization

Krishnan, P.G.; Wang, C.; Grady, K.A.
South Dakota State University; Department of Nutrition, Food Science and Hospitality; Brookings, SD 57007
Equipment Grant; Grant 99-35501-8254; $38,250; 1 Year

The purpose of this grant is to acquire a gas chromatograph mass spectrometer for the purpose value-added research in oilseeds. This instrument will accurately identify and measure constituents found in agricultural crops that have an economic impact. Constituents such as fatty acids, antioxidants, phytosterols, cell membrane lipids, tocopherols, and ferrulic acid occur at extremely small levels but nevertheless, exert significant effects. Many of these compounds have the ability to lower risk factors for major diseases such as coronary heart disease and some forms of cancer. Conventional processing conditions have reduced the recovery of such compounds. Alterative processing conditions are being studied. Knowledge of the natural variability of such constituent in plants is important to human health and economics of food production. Variability under the influence of environmental stress is also a focus of the research. The screening of sunflower, oat and soybean varieties for desirable fatty acid traits is directly applicable to oilseed processing and production of shelf-stable food products. This instrument will be used in the development genetically engineered high-oleic soybean lines and selection of mid-oleic and high-linoleic sunflower varieties. A further investigation involves the characterization of cell membrane lipids in freeze-tolerance and seed dormancy. The multi-disciplinary *use of* tile GC/MS will have positive implications for farmers, food processors, plant breeders, and plant physiologists.

9900595 Physiological Function of Maize Chloroplast Protein Synthesis Elongation Factor EF-Tu in Possible Relation to Thermotolerance.

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University of South Dakota; Department of Biology; Vermillion, SD 57069-2390
Strengthening Award; Grant 99-35100-8550; $100,000; 2 Years

The objective of this research is to understand the mechanism of action of maize chloroplast protein synthesis elongation factor EF-Tu (EF-Tu) in possible relation to thermotolerance. Previous work has shown that a heat tolerant maize line, ZPBL 1304, synthesizes increased amounts of EF-Tu under heat stress conditions, and that the chloroplasts of this line display increased thermostability. The increased synthesis of ZPBL 1304 EF-Tu associates with the heat tolerance phenotype, and in bacteria EF-Tu acts as a molecular chaperone (increases the refolding of unfolded proteins, protects proteins from thermal damage, forms complexes with unfolded proteins) and plays an important role in heat tolerance. Bacterial and maize EF-Tu-s are strikingly similar, and it is possible that EF-Tu in ZPBL 1304 line confers heat tolerance by acting as a molecular chaperone. The work described in this proposal is designed to test this hypothesis. Studies will be conducted to investigate whether EF-Tu acts as a molecular chaperone and helps chloroplasts and/or other cell proteins withstand high temperatures. The effects of EF-Tu on thermostability of enzymes and chloroplast proteins, and the effects of EF-Tu on thermal damage of cell extracts will be examined. In addition, the steady state levels of EF-Tu mRNA will also be measured under normal and heat stress conditions to determine if EF-Tu may be regulated at the level of transcription. The studies outlined in this proposal will provide additional information on the fundamental role of maize EF-Tu. Our results will be of importance in creating new maize strains with greater heat tolerance.

9903626 Metabolic Regulators and Intermediates in Cattle on Tall Fescue

Browning Jr., R.
Tennessee State University; Cooperative Agricultural Research Program; Nashville, TN 37209-1561
Seed Grant; Grant 99-35208-8326; $49,615; 2 Years

Tall fescue is a widely used pasture grass often infected with *Neotyphodium coenophialum,* a fungal endophyte. Cattle grazing endophyte-infected tall fescue can have reduced growth and reproductive rates resulting in an estimated $600 million to $1 billion of lost income annually. Short term exposure of cattle to ergotamine, a compound representative of ergopeptine alkaloids produced by *N. coenophialum* and isolated from endophyte-infected tall fescue, causes physiological and endocrine changes that correspond with poor performance. Ergotamine altered blood levels of metabolic hormones (e.g., cortisol, insulin, glucagon) and metabolites (e.g., glucose, urea nitrogen, triglyceride). These responses suggest reasons for reduced cattle productivity caused by the endophyte. The goal of this research is to determine if altered hormone and metabolite levels observed after ergotamine infusion occur when cattle consume tall fescue over a period of weeks. Steers will be fed endophyte-infected or non-infected tall fescue and bled periodically to measure 1) basal concentrations of hormones and metabolites involved with metabolic processes and 2) hormone and metabolite responses to an acute nutrient challenge. Basic mechanisms underlying the diminished growth and reproduction of cattle consuming endophyte-infected tall fescue are not defined. Findings from this project should provide insight and direction for future initiatives aimed at identifying specific pathways by which endophyte-infected tall fescue and associated ergot alkaloids reduce productivity. A better understanding of these mechanisms will aid in devising adequate strategies for use by cattle producers to minimize production and economic losses as a consequence of feeding endophyte-infected tall fescue.

9903638 Demographic and Geographic Information Modeling System

Busby, M.R.
Tennessee State University; Center of Excellence, Information Systems; Nashville, TN 37209-1561

Equipment Grant; Grant 99-35402-8253; $25,250; 1 Year

This equipment grant supports the implementation of a Demographic and Geographic Information (and Visualization) Modeling system (DGIVMS) at Tennessee State University to underpin collaborative research among a wide range of disciplines based on common, well-established databases. The system will apply to a diverse array of research in agriculture (economics, food distribution, weather, forestry, digital maps, workplace preparation, urban sprawl), history and geography, (GIS, GPS, mapping, remote sensing, archeology with visualization); computer science and information systems (telecommunications, mapping, simulation), engineering (industrial and civil), as well as education and sociology (demographic analysis, workforce preparation, workforce diversity). These widely diverse applications share common GIS or other demographic databases that can be interleaved by the DGIVMS in virtual modes among many disciplines, to include classroom work by undergraduate and graduate students.

The DGIVMS will underpin significant growth in research competitiveness at TSU from the introduction of leading edge computational tools and resources, particularly in the *social sciences and other non-science disciplines. It* will introduce all TSU faculty and students to new areas of research in the environment, food production, forestry research, and the social sciences. Applications of the DGIVMS could eventually include about 70% of the TSU research faculty.

The equipment, a fully-integrated system, consists of six (6) Pentium III desktop workstations; one (1) Xeon Pentium III server unit; one (1) mass storage server unit for sharing large GIS files; one (1) high-end visualization workstation; ten (10) handheld GPS units, and three (3) 20-unit network licenses for GIS, visualization, and collaboration software (“groupware"). The DGIVMS will be configured as a local "intranet” linked to the TSU Internet backbone.

9901014 Pesticide Fate and Removal in Constructed Wetlands

Stearman, G.K.; George, D.B.; Weathers, L.; Spratt, H.
Tennessee Technological University; Center for the Management, Utilization and Protection of Water Resources; Cookeville, TN 38505

Strengthening Award; Grant 99-35102-8523; $110,000; 2 Years

Growing nursery stock in containers under highly controlled conditions has been a practice increasing in popularity in the United States. An environmental consequence of containerized plant production is the fertilizer and pesticide-laden runoff generated by frequent and intense irrigation. Seventy to eighty percent of the water applied to a container crop by overhead irrigation falls between the pots or in the aisles and is lost to runoff. Research shows that bedcover composition is a significant factor in herbicide movement after application and irrigation, and runoff quantity from nurseries has increased with synthetic (fabric or plastic) or packed clay groundcovers.

Herbicides are applied in granules or by broadcast spraying, which is the most popular method. With containers spaced 30 cm apart, as much as 80 percent of broadcast applications fall on the bed cover instead of containers. Frequently, herbicides are sprayed directly on the groundcover to prevent weed growth, and with overhead irrigation typically following herbicide application, the granules or spray residues are carried with the runoff water. Aquatic organisms, wildlife, non-targeted vegetation, and humans can potentially be negatively impacted by contact or ingestion of these herbicides.

Research conducted by Tennessee Technological University has shown that subsurface constructed wetland systems can potentially remove herbicides in nursery runoff. Based on previous research, the proposed study will determine the herbicide removal processes and develop design equations or guidelines.

9903636 Development Potential of Immature Oocytes after Exposure to Various Stressors

Schrick, F.N.
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Equipment Grant; Grant 99-35208-8402; $28,308; 1 Year

Several environmental factors such as fescue toxicosis, mastitis and poor nutrition decrease reproductive performance in beef and dairy cattle; in part, through reduced embryonic development. Increased embryonic and fetal loss of cloned embryos decreases efficiency of somatic cell nuclear transfer to produce live offspring. Causes of these anomalies in embryonic development are unknown, but the ability of continually collecting oocytes from animals affected by these factors would allow studies to be initiated at the “root” of the problem in terms of developmental potential of oocytes. Specific objectives of the studies which would utilize oocyte aspiration via ultrasonography include: 1) to determine effects of nutritional restriction, fescue toxicosis, and clinical mastitis on the ability of oocytes to fertilize and continue normal development; 2) to identify individual donor animals that produce morphologically normal oocytes that differ in their developmental potential; 3) to determine if reduced development potential of immature oocytes obtained from individual donor animals is due to cytoplasmic or genetic differences within the oocyte; and 4) to examine effects of reconstructing cloned embryos in maternal (oocytes from the donor animal) versus nondescript foreign (oocytes from an unknown animal) cytoplasm. These research focuses will provide important information relating to embryonic development in cattle

associated with reduced nutrition, fescue toxicosis, mastitis, and somatic cell nuclear transfer. With data obtained from these studies, related studies will be initiated to gain insight on possible mechanisms causing early embryonic loss and reduced pregnancy rates in beef and dairy cattle.

9903644 Microscopic Evaluation of Tissue Health and Reproductive Competence in Farm Animals

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University of Tennessee; Department of Animal Science; Knoxville, TN 37996

Equipment Grant; Grant 99-35208-8540; $25,104; 1 Year

Experiments and justification for purchase of an upright microscope with epi-fluorescence and photography capabilities are described. Laboratory interests center on health and reproductive effects of fungal mycotoxins on bobwhite quail, with secondary efforts directed at the effect of immune system proteins (tumor necrosis factor alpha) on reproductive function in the laying hen. Declining populations of wild birds in the Southern US, plus correlation of cool, wet, Spring weather to lower fall populations of wild quail have led to the hypotheses that toxic fungal growth on over-wintered soybeans may be partly responsible for declining quail numbers.

Cytokine proteins, products of the immune system, act as local regulators of cellular function. Commercial chickens raised in high density, enclosed structures may potentially suffer from immune suppression and decreased reproductive performance due to over production of cytokine proteins. The proposed Nikon E800 microscope will be used to: 1) evaluate tissue and blood changes in bobwhite quail exposed to T-2 mycotoxin; 2) evaluate the ability of chicken immune cells to illicit cellular defense mechanisms in the presence of T-2 toxin; and 3) study reproductive cell function in laying hens exposed to the cytokine protein tissue necrosis factor alpha. Vastly increased analytical and documentation capability will be made possible through purchase of this microscope.

9903637 Equipment Request for Image Analysis Software

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Equipment Grant; Grant 99-35208-8542; $15,518; 1 Year

Improvements in animal agriculture such as increasing reproductive efficiency in livestock species will have long range impacts on U.S. agriculture. Success will be dependent in part on gaining an understanding of the mechanisms involved in growth and differentiation of early embryos. Use of equipment as required for computer-assisted image analysis will improve the ability to obtain objective and quantitative assessments of experimental endpoints. Intended uses for requested equipment are to acquire color and fluorescent images of early bovine embryos for more precise estimates of gene expression and to identify transgenic embryos. Moreover, this equipment will be invaluable for investigating whether calcium influxes following exposure to PGF_2 or other stressors induce embryonic death. The diversity of applications of computer-assisted image analysis make it usefu1 to study other areas of interest to U.S. agriculture. Specifically, the requested equipment will aid in: 1) analysis of natural microflora, and intestinal pathogens on the development and integrity of the GI tract in young pigs; 2) investigation of the role of peptide transport proteins in seed development; 3) ascertaining the distribution and topology of membrane proteins in order to assist in drug design and delivery; and 4) studying RNA-protein interactions involved in assembly of southern bean mosaic virus (SBMV). Computer-assisted image analysis will greatly facilitate the completion of research important to U.S. agriculture.

9903597 Expression of Yeast Glycogen Biosynthetic Genes in *Arabidopsis*

Gibson, S.I.
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Seed Grant; Grant 99-35311-8683; $50,000; 2 Years

Plant starches constitute the major source of calories consumed directly by humans as well as by animals raised for human consumption. In addition, vast quantities of starches are used in the paper, textile and packaging industries. Despite the critical importance of starch to agriculture, fundamental gaps in our knowledge of starch synthesis remain. This lack of detailed information regarding starch synthesis is an impediment to using genetic engineering to alter starch production in plants. In contrast, information regarding glycogen synthesis is relatively complete. Glycogen, which is produced by organisms ranging from bacteria, to yeast, to humans, is chemically almost identical to starch. The major goal of this project is, therefore, to test the feasibility of using the information and tools gained from studies on glycogen synthesis to increase starch quantity or quality in plants. Specifically, yeast genes encoding proteins involved in glycogen synthesis will be cloned into plant expression vectors. These plant expression vectors will then be used to transform the model plant *Arabidopsis thaliana.* The starch produced by these transgenic plants will be analyzed to determine which combinations of yeast glycogen biosynthetic genes are most effective in causing economically useful alterations in starch production. In the future, the information gained from these experiments will be used to design the most effective approaches for using yeast glycogen biosynthetic genes to improve starch quantity or quality in crop plants.

9903612 Purge & Trap Concentrator for Air Quality and Odor Research at CAFOs

Parker, D.B.; Williams, D.L.
West Texas A&M University; Division of Agriculture; Canyon, TX 79016

Equipment Grant; Grant 99-35208-8249; $24,822; 1 Year

More than seven million beef cattle are grown annually in the Southern High Plains area, and some of these feedlots have as many as 80,000 animals in one location. The control of odors and air quality at feedlots has become a growing concern in the past few years. Because the concentration of organic compounds contributing to odor and air quality issues at feedlots are too small to be measured with conventional laboratory equipment, specialized equipment is necessary to concentrate large volumes of organic compounds into small volume, large concentration samples where they can then be measured. This research grant will fund the purchase of a scientific instrument called a "purge and trap concentrator." Research projects in which the instrument will be used will include an evaluation of chemical amendments to reduce gaseous emissions from feedlot surfaces, and measurement of odor producing gases resulting from the decomposition of manure. The instrument will be used by research personnel located at West Texas A&M University in Canyon, TX, Texas A&M University Research and Extension Center in Amarillo, TX, and USDA-ARS in Bushland, TX.

9901798 Asymmetric Cytokinesis in *Pelvetia compressa*

Kropf, D.L.
University of Utah; Department of Biology; Salt Lake City, UT 84112

Strengthening Award; Grant 99-35304-8230; $100,000; 2 Years

Cell division is fundamental to growth and development in all organisms. However, the role of cell division in determining the shape (morphology) of animals and plants is very different. Animal cells are mobile and the shape of an animal depends on how cells arrange themselves. In contrast, plant cells are surrounded by a wall and cannot move. Morphology in walled organisms is determined by two main factors, the orientation of cell division and the direction of cell elongation. In this award, we will investigate the mechanisms that control cell division in plants. The experiments will be conducted using fertilized eggs of marine brown algae because we can get large numbers of these cells quickly and they undergo a precise pattern of cell divisions in early development. We will investigate two important questions: 1) How is the orientation of division chosen? and 2) What are the components of the cellular machinery that actually divides the cell? From this work, we hope to gain insight into how plants control their shape during development.

9903541 Behavior and Olfaction of Courtship Pheromones in Female Heliothine Moths

Vickers, N.J.
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Seed Grant; Grant 99-35311-8373; $46,115; 2 Years

Many behaviors performed by animals are modulated by their sense of smell. The ability of male moths to locate females upon the basis of their keen olfactory sense has been well studied. When the male alights near the female, the olfactory dialog continues in the form of a courtship display In comparison to our understanding of male behavior and olfaction, we know relatively little about the odors and odor-mediated behavior of female moths, even though olfaction apparently plays an important role in mate-selection and subsequent location of suitable host plants for egg-laying. We will investigate the olfactory nature of courtship communication in three closely-related species of Heliothine moth. Once the odors released by specialized male courtship structures have been determined and their significance to females tested, neurophysiological recording) from the olfactory centers will be performed to understand how behaviorally-relevant odor signals are processed and represented by neuronal activity in the female brain. Moths have relatively simple and accessible olfactory systems which have essentially the same anatomical arrangement as those found in a wide diversity of other organisms. Studies on moths therefore provide us with an opportunity to further our understanding of the sense of smell in general. Furthermore, the comparative nature of the proposed study includes two serious agricultural pests and therefore offers the possibility of exploiting the reliance of these animals upon their sense of smell to develop novel, environmentally-rational strategies for their control.

9903732 Plant Growth Facility
Sieburth, L.E
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Equipment Grant; Grant 99-35311-8421; $48,644; 1 Year

Three plant growth chambers will be purchased to allow precise temperature controlled plant growth. Growth at precise temperatures is required to investigate the roles of specific genes in plant development. Two research projects require growth at controlled temperatures. The first project is an investigation into the roles of specific genes in controlling vein pattern, and the second project is an investigation of the functions of flower developmental control genes. Plant veins function to transport water and sugars throughout the plant. Vein pattern is believed to allow these transport functions to proceed efficiently, however molecular mechanisms that control vein pattern are still not understood. We have identified vein pattern genes genetically through the isolation of vein pattern mutants. Many of these mutants show temperature specific growth requirements. The plant growth chambers will be used to grow plants under precise temperatures for phenotypic characterizations and for temperature shift experiments designed to identify the developmental time at which each gene is required. The second research project seeks to understand the functions of flower developmental control genes. Most research focuses on how the *AGAMOUS* gene functions to specify stamens, the pollen-bearing flower organs, and carpers, the seed-producing flower organs. Both of these projects have potential long term applications to agriculture because they will allow us to identify and understand the functions of specific genes in plant development. A clear understanding of the developmental roles of these genes is essential before they can be used for biotechnology applications.

9903666 Acquisition of a Protein Sequencer to Enhance Agricultural Research
Grover, T.A.
Utah State University; Biotechnology Center; Logan, UT 84322-4700
Equipment Grant; Grant 99-35208-8127; $50,000; 1 Year

The objective of this proposal is the acquisition of a Protein Sequencing System to upgrade the research support offered to scientists by the core service labs of the Utah State University Biotechnology Center. Several ongoing projects at Utah State University will benefit from the acquisition of a protein sequencer. These include the development of a rapid antibody based detection method for the whirling disease parasite in infected trout and rivers; identification of the peptides that cause bitterness in cheese; identification of antigenic sites on the viruses that causes Bluetongue disease in sheep for the development of diagnostic assays and novel vaccines; unraveling the genetic mechanisms responsible for the antifungal activity or syringomycin, a peptide produced by the wide-spread plant bacterium *Pseudomonas Syringae;* characterization of protein, some found in bovine whey, that play a role in the development, maintenance, and repair of the nervous system and the characterization of specific metabolic enzymes from the soil bacterium, *Xanthobacter*, responsible for hydrocarbon breakdown. The acquisition of a protein sequencer will allow the investigators in these projects to pursue research questions and future funding in areas that are directly applicable to the goals of the NRICGP.

9901871 Phosphoinositide Signaling in Plants
DeWald, D.B.; Hama, H.
Utah State University; Department of Biology; Logan, UT 84322-5305
Strengthening Award; Grant 99-35304-8114; $100,000; 2 Years

Intracellular signaling molecules can regulate eukaryotic cell growth and stress-response. This research focuses on how these signaling molecules control plant cell growth and stress-response. Members of a class of intracellular plant signals called phosphoinositides have an inositol ring head group and two fatty acid tails. These molecules reside in cellular membranes and, we hypothesize that when the inositol head is modified via the addition of phosphate groups, specific phosphoinositides can bind to regulatory proteins and alter their cellular location and/or activity. In addition, a metabolite of one of the phosphoinositides apparently causes calcium levels to increase dramatically inside cells. This research will explore how phosphoinositides and phosphoinositide metabolites serve as signaling molecules in the simple plant, *Arabidopsis thaliana.* We will examine the quantity and molecular species (there are 7 types) of phosphoinositides found in plant tissues during normal growth and under stress conditions (e.g., high salt growth conditions and elevated growth temperatures). Phosphoinositide-binding proteins will be isolated and we will examine whether calcium levels change in cells with high concentrations of specific phosphoinositides. Altogether, these efforts will help us understand how plants utilize membrane phospholipids to control growth and their response to stress. This basic information could potentially be used to engineer plants that are more resistant to stress.

9903743 Telomerase Activity and Telomere Length in Bovine Oocytes, Embryos, and Clones

Reed, W.A.
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Seed Grant; Grant 99-35208-8547; $32,669; 2 Years

Dolly, the now famous sheep cloned from adult cells, has recently been reported to have abnormally short telomeres for her age. (Telomeres are structures on the end of each chromosome that protect the genetic code from degradation during each cell division.) Other research has demonstrated that shortening of telomeres can prevent subsequent cell division and trigger programmed cell death. Telomerase, the enzyme responsible for maintaining telomere length, is generally inactive in adult cells, but it must be turned on during either gametogenesis or early embryogenesis to restore telomeres to their full length as part of the nuclear reprogramming that must be completed for normal development. The efficiency of the cloning process depends upon correct reprogramming of the donor nucleus. Animal cloning to date has assumed that factors in the egg will correctly reprogram the donor nucleus but little is understood about the actual process. The current knowledge of telomerase activity during early development comes from limited research using naturally produced rat and human embryos and eggs. There is little agreement as to the timing of telomere reprogramming between these species. The proposed experiments are designed to investigate telomere length and telomerase activity and the timing of telomere reprogramming as a component of nuclear reprogramming in naturally produced and cloned cattle embryos. The information gained from these experiments may have a dramatic impact on the efficiency of cloning by nuclear transfer not only in cattle but also in other species and will further basic knowledge of nuclear events during embryogenesis in mammals.

9901085 Quantifying the Exposure of Streams to Sediment Inputs from Managed Forests, a Risk-based Approach

Pack, R.T.; Tarboton, D.G.; Benda, L.
Utah State University; Department of Civil and Environmental Engineering; Logan, UT 84322-4110

Strengthening Award; Grant 99-35102-8599; $279,500; 3 Years

This project will develop and test a risk-based management tool for quantifying the potential frequency and magnitude of sediment input to streams in managed forests. This work will emphasize the characterization and integration of the frequency-magnitude character of processes that combine to contribute sediment to stream channel networks. A key objective is to adapt existing deterministic basin-scale upland sediment models for use in a comprehensive risk-based framework. Formal risk analysis techniques are highly developed in other disciplines and provide an ability to take into account uncertainty in model parameters, and characterize the variability inherent in the processes being modeled. Chains of events including climatic triggers that lead to landslide and erosion events that in-turn lead to sediment transport and input to streams will be characterized across the landscape. The effects of managed forests in modifying the governing variables and altering the frequency magnitude curves of sediment input will be estimated for use in making forest management decisions. The testing of this approach will be conducted within an area that has been extensively documented as having severe sediment impacts on streams in central Idaho. This work will directly address the need for research to increase our understanding of forest ecosystems and management practices to optimize our stewardship of forest ecosystems. It also addresses the need to reduce the release of waterborne contaminants of agricultural origin.

9903451 Seasonal Patterns of Root Growth and Carbohydrate Partitioning in Asparagus

Drost, D.T.
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Sabbatical Grant; Grant 99-35311-8116; $48,137; 1 Year

Asparagus is one of the most important perennial vegetables grown in the world. While top growth is well understood, access limits our understanding of the growth patterns of the root system. Scientists agree that large tops produce more carbohydrates. These are stored in the roots for later use by the developing spears. Asparagus accumulates fructans as the major carbohydrates, but more research is needed to quantify their changes over time and where they are stored in the root system. This study will; 1) monitor the changes in root density over time in field grown asparagus, 2) measure the changes in specific fructans during these periods and 3) assess the carbohydrate changes in roots collected from water stressed asparagus. Root samples from normal and extended (stressed) harvest plantings will be collected from spring to fall. Roots will be collected from different depths and locations of the root system at each harvest. Root number, length and dry weight will be measured. Storage roots will be measured for seasonal changes in total and specific fructans. Fructans will be extracted in water, filtered, and separated by high-pressure liquid chromatography into short and long chain sugars. Similar analysis will be conducted on root samples from water stressed asparagus. This information will be used to assess growth and productivity differences between stressed and non-stress asparagus. With improved knowledge of carbohydrate storage and root growth, cultural practices can be developed to optimize whole plant growth. This should help increase the productivity and profitability of the asparagus industry.

9903537 Genetic Limits to Ecological Range Expansion of Cattails

Stratton, D.
University of Vermont; Department of Botany and Agricultural Biochemistry; Burlington, VT 05405
Seed Grant; Grant 99-35106-8674; $49,996; 2 Years

Invasion of habitats by weeds or introduced species is a threat to natural habitats and as well as managed agricultural land. Predicting the ability of plant species to expand their range requires an understanding of the forces of selection that operate at current ecological boundaries. One explanation for the limits to range expansion assumes tradeoffs in performance in different habitats. Another assumes conditional expression of deleterious mutations at different loci in different environments. Our recent ability to identify regions of the genome that cause differences in performance across environments now allows us to ask fundamental questions about the limits of ecological range expansion. Two species of common cattail, *Typha latifolia* and *Typha angustifolia*, are dominant components of wetland communities in North America. Cattails represent an unusually well-studied system where the environmental controls on distribution are easily manipulated. Plants from the two species will be experimentally crossed to form an F_2 mapping population. We will then identify molecular markers that are segregating in the F_2 and use those to construct the genetic map. Finally, we will grow clonal replicates of the F_2 plants in two water depths to identify markers that are linked to regions that affect the relative habitat requirements of the two species. The mapping population will be clonally propagated and maintained as a permanent resource, to be used in future experiments addressing the genetic basis of submergence tolerance, the forces of selection that act at the ecological margin, and the evolutionary limits to range expansion in these species.

9903253 Regulation of Amino Acid and Peptide Transporters in Ruminant Intestine

Knapp, J.R.
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Strengthening Award; Grant 99-35206-8044; $182,000; 2 Years

Nutrient utilization by the whole animal is a function of nutrient transport and metabolism by the epithelial cells lining the gut epithelium. The transport systems and metabolic activity of these cells have a major impact on whole body energy expenditures, protein turnover, and nutrient availability for other body tissues. Not only is an understanding of the basic processes at the epithelial level critical for efficient energy and protein utilization, but it is also key to protecting the environment. One of the largest issues facing animal agriculture is management of manure nutrients. Protein nutrition and amino acid utilization are critical to both growing and lactating cattle. The levels and activities of amino acid and peptide transporters in the small intestine of ruminant animals may be different from those of non-ruminants because of different flow rates of digesta into the small intestine, lower digesta pH for prolonged transit times, and the inclusion of microbial protein in ruminant digesta. The hypothesis to be tested in this proposal is that the structures and regulation of amino acid and peptide transporters in the small intestine of ruminants are similar to monogastric animals. A better understanding of how dietary and microbial proteins are digested and absorbed in ruminant livestock will lead to improved ration formulation, optimization of nutrient use for animal production, and reduced manure nutrient load released into the environment.

9900913 Microbial and Physico-chemical Control of Nitrogen Mobility in Forest Ecosystems

Ross, D.S.; Lini, A.
University of Vermont and State Agricultural College; Department of Plant and Soil Science; Burlington, VT 05404
Strengthening Award; Grant 99-35101-8235; $100,000; 2 Years

Atmospheric deposition of nitrogen on forest ecosystems, from industrial and automotive emissions, has been linked to the acidification of lakes and streams. Understanding what factors control the movement of nitrate through the forest ecosystem is essential to an understanding of the impacts of deposition. Previous work has shown that the nitrate that reaches streams is derived from soil microbial processes, not directly from deposition. This study will focus on the forest soil microbial population and how it transforms nitrogen. Additionally, it will compare a number of established and new techniques to estimate the rate of nitrogen transformations in forest soils of the northeast United States. The stable isotopes of nitrogen and oxygen in nitrate will be measured to determine the source and fate of nitrogen as it moves through the ecosystem. Molecular biology approaches will be used to characterize the microbial community with special attention to the ammonia oxidizing population (the group that produces nitrate). The relationship between the microbial community and the rate of nitrate production will be assessed. Results should provide valuable information on the role of microbial community dynamics in watershed nitrogen mobility and provide insights on the mechanism of increased acidification from increased nitrogen deposition.

9903530 Phenotypic Plasticity or Genetic Specialization in an Invasive Grass

Molofsky, J.
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Seed Grant; Grant 99-35106-8270; $50,000; 2 Years

Invasive species are changing ecosystems around the world. It is essential for us tc understand the characteristics that enable a plant to take over natural communities. Why a species becomes a pest in some areas and not in others may lie in the interactions between its morphological characteristics and the physical and biotic environment it encounters. Reed canary grass, *Phalaris arundinacea, is* a tall, clonal, cool-season perennial grass. It was originally introduced into the U.S. as a forage crop, but has since escaped domestication and spread into wetland and pasture communities. Reed canary grass exhibits variation in its aggressiveness. The objective of my research is to determine the characteristics that make reed canary grass an aggressive invader and how these vary among genotypes and environmental conditions. By comparing successful genotypes with unsuccessful genotypes (as determined by survival and growth measurements (biomass, number of tillers, and biomass allocation patterns)) across a range of different environments, I will determine how the expression of traits associated with "aggressiveness" varies in different environments and with different genotypes**.**

9903594 Effects of Excess Nitrogen and Post-Harvest Recovery on Plant Nutrient Dynamics

May, J.D.; Gilliam, F.S.
Marshall University; Department of Biological Sciences; Huntington, WV 25755

Seed Grant; Grant 99-35106-8399; $49,978; 2 Years

There is increasing evidence that significant forested regions of the US and Europe are becoming nitrogen (N) saturated as a result of chronic, excessive N deposition from the atmosphere. To date, research has focussed on effects of N saturation on soil processes, including its role in acidifying the soil and in leaching (removing) nutrient cations, e.g., calcium, magnesium, and potassium. Little attention has been given to related responses in the dynamics of plant nutrients that might ameliorate the altered soil nutrient conditions. This proposal seeks Seed Grant funding to study (1) the effects of chronic N fertilization on foliar dynamics of N, phosphorus, potassium, calcium, and magnesium in an eastern deciduous forest; and (2) the response of foliar nutrient dynamics during recovery from whole-tree harvesting on plots receiving N and/or cation fertilization treatments. A primary focus of the work will be to assess changes in patterns of nutrient resorption—the internal reclamation of foliar nutrients that takes place in autumn, just prior to autumn leaf abscission. Because resorption efficiencies can increase in response to imposed nutrient stress, resorption may provide an internal conservation mechanism by which plants can partially offset the effects of nutrient limitations caused by leaching of cations from N-impacted forest soils. Furthermore, if resorption responses are significantly altered, they may offer a measure of the degree of nutrient limitation being experienced by individual plants, and thus a more realistic picture of nutrient stress than can be determined from soil characteristics alone. If so, they would offer an additional measure of forest health in both managed and unmanaged forests.

9903593 Transformation and Regeneration of Wetland Monocots for Phytoremediation

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Seed Grant; Grant 99-35106-8180; $49,928; 2 Years

Urbanization, industrialization and agricultural activities have lead to extensive pollution problems. Cleanup of existing hazardous wastes' by conventional technologies, is expensive. The objectives of this project include developing genetic engineering protocols for Cattail and Bulrush cells, regenerating and characterizing the engineered plants, and generating cells of these plants expressing genes for mercury resistance and remediation. The long-term goals of this research are to create wetland plants engineered to remove and process heavy metals from polluted waters The common wetland plants Cattail and Bulrush have been used for decades in wetlands for the treatment of polluted water. The availability of genes with specific remediation activities, coupled with gene transfer systems, offer the possibility of creating plants with tailored remediation capabilities. Model terrestrial plants have been transformed with modified bacterial genes conferring the ability to metabolize and detoxify organic mercury. This suggests Cattail and Bulrush, engineered with such genes? could be used for transporting mercury from contaminated aquatic sediments. No genetic engineering and regeneration systems, however, have been developed for these plants. This presents a barrier to the evaluation of the ecological benefits of phytoremediation activity towards metal pollution of aquatic systems. Goals of this project are to develop genetic engineering protocols, using an engineered bacterium. *Agrobucterium,* to insert foreign genes, and to regenerate and characterize transformed plants. furthermore, to create and characterize engineered cells expressing modified bacteria] genes conferring the ability to metabolize and detoxify mercury. Long-term goals are to generate and characterize transformed plants expressing genes for mercury remediation.

9903716 Acquisition of a Waters HPLC to Support Fungal Biology Research

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Equipment Grant; Grant 99-35106-8242; $30,021; 1 Year

Symbiotic associations are critical in enhancing the stress resistance of higher plants. One such association, formed between mycorrhizal fungi and the roots of many tree species, plays a critical role in increasing the ability of trees to resist toxic metals in soils. These fungi produce compounds that detoxify metals in the soil. A second symbiotic association, formed between endophytic fungi and various grasses, benefits the growth and vigor of the host plant, but poses various problems to agricultural systems relying on grasses for forage. These fungi produce compounds that are toxic to grazing animals. To better understand the role of these symbiotic associations in natural and agronomic ecosystems, several research groups at West Virginia University focus on the physiology and biochemistry of plant-microbe interactions. The objectives of these programs are to identify the mechanisms of metal tolerance in ectomycorrhizal fungi and isolate metal tolerant fungi for use in reclamation efforts, and to identify and develop endophytic fungi that confer stress tolerance to host plants but exhibit reduced production of toxins in forage. This funded project will strengthen and extend these ongoing programs in fungal biology at West Virginia University by supporting the purchase of a high performance liquid chromatograph that allows the detection and analysis of compounds produced by mycorrhizal and endophytic fungi. These efforts will enhance agriculture by potentially identifying mycorrhizal fungi that can be used in the reclamation of metal-contaminated soils and endophytic fungi for use in pasture production systems.

9903450 Natural Enemy Control of Insect Herbivores in Diverse Agricultural Landscapes

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Sabbatical Grant; Grant 99-35311-8569; $53,953; 1 Year

The expected outcome of this sabbatical project is to increase my knowledge in evaluation of plant, herbivore, and natural enemy attributes that govern their interactions within diverse agricultural landscapes, and to incorporate existing information on small grain - aphid - parasitoid interactions into modeling Russian wheat aphid population dynamics in the western Great Plains. By participating ~n ongoing research projects with host scientists (Drs. J.C. van Lenteren and N.C. Elliott) at their facilities (Wageningen Agricultural University and USDA ARS Plant Science Laboratory, respectively), the following sabbatical research objectives will be pursued:

Wageningen: acquire expertise in using specialized equipment to assess mechanisms governing natural enemy - herbivore interaction in diverse plant systems, and participate in companion field studies to link mechanisms with their function in multicropping systems.

USDA ARS: learn pertinent applications of GIS/ modeling approaches for incorporating landscape information with information on natural enemy -herbivore interaction, and ground-truthed remotely sensed data for use in Russian wheat aphid spatial population dynamics modeling.

The tools acquired and approaches learned will contribute to establishment of a research program that broadly aims to understand herbivore regulation in diverse landscapes and predict the ecological conditions that contribute to herbivore regulation, and more specifically aims to better understand natural enemy and vegetative factors that contribute to Russian wheat aphid regulation. This approach is particularly desirable in the western Great Plains where cropping systems are highly associated with native/near native habitat (grasslands), and agricultural economics are such that there is a desire to diversify croplands.

9903628 Balancing Postruminal Amino Acid Supply

Hess, B.W.
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Seed Grant; Grant 99-35208-8559; $49,994; 2 Years

Erroneous predictions of amino acid supply to the small intestine are possible if cattle are consuming sub-optimal levels of forage. Supplemental protein sources resistant to degradation in the animal's stomach, the rumen, can be fed to balance supply of amino acids to the small intestine. However, unspecified amounts of supplemental protein may be fermented within the rumen when cattle are fed sub-optimal levels of forages. The objectives of the proposed research are 1) to develop a model to predict the flow of amino acids to the small intestine of cattle fed sub-optimal levels of forage; 2) to evaluate a protein supplement formulated to balance flow of amino acids to the small intestine of cattle fed sub-optimal levels of forage, and 3) to adjust the quantity of supplemental protein to account for additional ruminal fermentation of feed protein when cattle are fed sub-optimal levels of forage. Increased knowledge of the level and pattern of amino acids entering the small intestine of cattle fed various levels of forage is critical to the optimization of amino acid requirements of cattle. Optimization of amino acid requirements should enable producers to increase beef cattle production.

9902390 Influence of Dietary Oils on Reproduction in Primiparous Beef Cattle

276 Strengthening Program

Moss, G.E.; Hixon, D.L.
University of Wyoming; Department of Animal Science; Laramie, WY 82071-3684
Strengthening Award; Grant 99-35203-8040; $155,000, 3 Years

The largest reproductive loss experienced by beef producers is failure of cows to conceive before the end of the breeding season. Research indicates that dietary oil supplements may improve beef cow reproduction. Therefore, specific objectives of this project are to: 1)Determine if unique fatty acids in oil supplements are responsible for improved postpartum reproduction; 2) Determine how beneficial effects of oil supplements on reproduction are mediated; and 3) Determine if beneficial effects of oil supplements on reproduction could be due to the presence of estrogenic compounds. Successful completion of the project should enable producers to adopt management strategies which improve beef cow productivity.

POSTDOCTORAL FELLOWSHIPS

Postdoctoral Fellowships are available to individuals who have recently received their doctoral degree and are initiating an independent research program under the guidance of an established investigator. Awards are made directly to the individual and are primarily for salary support. Postdoctoral Fellowships are reviewed in the program area most appropriate for the type of research proposed; therefore, these nontechnical summaries also appear under the respective program areas.

9902469 Genetic Variation in Grape Phylloxera Life History Traits by Spontaneous Mutation

Downie, D.A.
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Postdoctoral Fellowship; Grant 99-35302-8404; $90,000; 2 Years

The grape phylloxera is a serious root-feeding pest of cultivated grapes worldwide. Reproduction in California is primarily, or completely, asexual. This means that the progeny of a given mother are genetically identical, barring the variation introduced by new mutations. The use of rootstocks that resist the effects of the insect has been the most effective method of control of the insect. Resistance will fail if genetic variation in pest populations is sufficient to allow adaptation to resistant rootstocks. Resistance of rootstocks has failed in the past. The extent of genetic variation incorporated into asexual lineages of grape phylloxera by new mutations is unknown. The work proposed will provide estimates of the rate of production of genetic variation by new mutations for the traits that are important in the control of the insect, and the effect of new mutations on insect reproduction and development. Lines of phylloxera will be asexually propagated and allowed to generate genetic variation by accumulating mutations for up to 50 generations, controlling for the influence of other factors that might produce variation. Periodically lines will be assayed for reproduction and development. The variation among lines, and the change in mean over the course of the experiments will allow estimation of the extent of genetic variation, its rate of production, and the effects of new mutations on reproduction and development. Knowledge of the extent, rate, and consequences of the origin of genetic variation by mutation in asexual lineages will help to generate more powerful predictions about the capacity of this insect to adapt to resistant rootstocks.

9902521 Characterization of a New Salicylic Acid-Binding Protein, SABP2

Slaymaker, D.H.
Rutgers, the State University of New Jersey; Waksman Institute; Piscataway, NJ 08854

Postdoctoral Fellowship; Grant 99-35303-8190; $70,000; 2 Years

Plants have an innate ability to defend themselves against disease-causing pathogens. However, pathogens are also programmed for survival. This battle between plants and their pathogens causes agricultural crop losses in excess of $100 billion annually. By genetically improving a plant's innate resistance mechanisms, agricultural crop protection can be improved. As for all technology-based advances, progress begins with a thorough research-driven understanding of the basic components of the system, here plant disease resistance.

Salicylic acid is a chemical component used by plants as an internal trigger for resistance against many plant pathogens. Three new plant proteins that interact with salicylic acid have been identified, and while all three proteins will be studied, this proposal focuses on one, SABP2. SAPB2 will be obtained in pure form from tobacco, which will allow identification of the protein and also allow the gene that encodes SABP2 to be cloned. Tobacco is used as a model plant because it is easy to grow and each plant provides a large amount of leaf tissue for research purposes. With the SABP2 gene in hand, we will learn the conditions under which the plant uses this gene, as well as the degree to which this gene directly effects plant disease resistance. These later studies will also be pursued in the model plant *Arabidopsis*. Once a though understanding of SABP2 function is obtained, the knowledge will be transferred to agricultural crops for developing biotechnology-based crop improvement strategies.

9900684 Plant-Based Food Intake and Cardiovascular Disease Risk With a Focus on Ethnicity and Gender

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Palo Alto, CA 94304-1583

Postdoctoral Fellowship; Grant 99-35200-7605; $89,982; 2 Years

National nutrition education campaigns emphasize a restriction of fat and cholesterol. Unfortunately, the low-fat, low-cholesterol (LFLC) educational method does not ensure a heart-healthy diet due to widely available processed low-fat low-cholesterol foods that are low in fiber and nutrients and high in sugars. Sugar-rich, low-fiber diets are commonly lacking in important nutrients and phytochemicals which are associated with a favorable cardiovascular disease (CVD) risk status. Little information is available on plant-based food intake (PBFI) in the US in relation to CVD risk factors, particularly among selected ethnic groups. Therefore, the applicant will study the role of PBFI in relation to CVD risk factors in over 20,000 African-American, Mexican-American, and White men and women using data from the Third National Health and Nutrition Examination Survey (1988-1994). It is believed that higher levels of PBFI will be associated with a favorable CVD risk status, independent of dietary saturated fat and cholesterol. CVD risk status will be based on several factors including blood cholesterol, blood pressure, obesity, diabetes, and blood nutrient levels. This project will provide valuable knowledge on what patterns of PBFI are associated with low CVD risk among selected ethnic groups and varying socioeconomic levels. Additionally, this knowledge will allow for the development of culturally specific educational tools to promote PBFI in accordance with USDA nutrition objectives which include increasing dietary intakes of fruits, vegetables, whole grains, and beans.

9901330 Cytomolecular Mapping in Sorghum and Maize Using In Situ Hybridization
Peterson, D.G.
University of Georgia; Department of Crop and Soil Sciences; Athens, GA 30602
Postdoctoral Fellowship; Grant 99-35300-7819; $90,000; 2 Years

The activity of a gene is determined not only by its DNA sequence, but also by its location on a chromosome. Consequently, an understanding of chromosome structure is prerequisite to understanding how genes function in living organisms. The most direct means by which genes can be positioned on chromosomes is by fluorescence in situ hybridization (FISH). In FISH a DNA marker associated with a gene is hybridized to chromosomes fixed on glass microscope slides. The chromosomal location of the marker (gene) is then visualized. We propose to use FISH to position markers at various places along a specific sorghum chromosome. The resulting "cytomolecular map" will be compared with the existing genetic map of this sorghum chromosome. If time permits, DNA markers used in constructing the sorghum cytomolecular map will be hybridized to maize chromosomes to construct cytomolecular maps for maize chromosomal regions sharing homology with the sorghum markers. This work will contribute directly to long-range improvement and sustainability of U.S. agriculture by providing (1) insight into the relationship between linkage distances, base pair distances, and chromosome structure in two important crop species, (2) a better understanding of the cytological and molecular factors affecting recombination, a process that is central to plant improvement, (3) means by which the site of a transgene insertion can be rapidly detected and characterized, (4) a better understanding of the evolutionary divergence of Sorghum and Zea, and (5) a foundation for further cytomolecular mapping in other crops.

9901784 Regulation of Triose Phosphate Export from Chloroplasts at Night
Laporte, M.M.
Iowa State University; Department of Botany; Ames, IA 50011
Postdoctoral Fellowship; Grant 99-35306-8649; $90,000; 2 Years

Understanding the partitioning of photosynthetic carbon among starch, sucrose and other products is of fundamental importance to the production of crop plants with desirable agronomic and nutritive traits. However, the pathway and regulation of leaf starch degradation at night has received less attention than other components of the system. For many years, it was thought that the products of starch degradation were exported from chloroplasts at night as triose phosphates, phosphorylated three carbon sugars, which are the primary export product from chloroplasts during the day. Recent evidence has made it clear that neutral sugars are the primary export products of starch degradation at night rather than triose phosphates. However, it remains unclear why triose phosphates are not exported from chloroplasts at night. To explore this question, I propose to increase the rate of triose phosphate production at night in chloroplasts of wild-type *Arabidopsis thaliana* and an *Arabidopsis* mutant that lacks chloroplast neutral sugar transport. In the mutant, the change in levels of intermediate compounds and rates of starch degradation in response to increased production of triose phosphates in chloroplasts in the dark will allow me to test specific hypotheses about where the limitation to triose phosphate export at night occurs. The response of wild-type to increased triose phosphate production at night will provide insight into the evolution of independent mechanisms for carbon export from chloroplasts in the light and the dark.

9900937 Nitrogen Fertilization Effects on Soil Organic Matter Dynamics in a Corn-Belt Agroecosystem
Russell, A.E.
Iowa State University; Department of Botany; Ames, IA 50011-1020
Postdoctoral Fellowship; Grant 99-35101-8591; $90,000; 2 Years

Human activities have altered the global carbon cycle in profound ways. Nitrogen fertilization is one agricultural practice that can influence soil carbon stocks and atmospheric inputs by altering the quantity and/or quality of carbon and nitrogen inputs from crop roots and aboveground residues. Nitrogen fertilization can affect losses of carbon and nitrogen from soil by influencing the breakdown of soil organic matter (SOM) to release CO_2. Relationships between fertilization rate and grain production are well-studied, but surprisingly, relationships between excessive fertilization, root production and CO_2 losses are poorly understood. The objectives of this research are to quantify effects of increasing rate of nitrogen fertilization on carbon inputs to, and losses from, soil. To determine effects on inputs, Russell will measure total annual aboveground crop growth and root biomass production in the top 60 cm of soil in experimental plots established four years ago (Ames, IA) in a corn-soybean rotation under three levels of N fertilization: 60, 120 and 180 kg/ha. She will determine fertilizer effects on tissue quality (C, N and lignin content) and decomposability and soil CO_2 losses to the atmosphere. Her approach will be to integrate field and laboratory experimentation with process-based modeling using the CENTURY model. This topic is highly relevant because, with so much land in the upper Midwest devoted to corn production, nitrogen fertilization effects that result in small annual decreases in SOM will contribute to soil degradation on a large scale, with important consequences to soil fertility, water resource quality and global biogeochemical cycles.

9902641 Larval Exposure to Juvenile Hormone and Insect Growth Regulators and Division of Labor in Honey Bee (*Apis mellifera*) Colonies

Elekonich, M.M.
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Postdoctoral Fellowship; Grant 99-35302-8187; $90,000; 2 Years

Pyriproxyfen is a synthetic mimic of the naturally occurring insect hormone, juvenile hormone (JH) and is used in many insecticides. Although pyriproxyfen does not kill adult honey bees, sublethal effects on adults and on developing bees (larvae and pupae) are not well known. Given the importance of pollination by honey bees to U.S. agriculture, it is important to assess potential effects of pesticides on the control of social behavior and development in honey bees. I have recently obtained data on fast and slow developing genetic groups of honey bees suggesting that faster developing groups (as measured by the onset of foraging behavior) have higher JH levels during two time points early in the larval period. These time points coincide with the onset of increasing JH which causes the totipotent female larva to differentiate into a queen. These results suggest that these time points may indicate a developmental window when exposure to JH or JH mimics like pyriproxyfen affect brain and behavior to influence how quickly a bee grows up to become a forager. I will test this hypothesis by treating larval bees with JH and pyriproxyfen and measuring their effects on behavioral development as measured by age at onset of foraging, sensitivity to different amounts of JH and pyriproxyfen; and effects of larval treatment with JH or pyriproxyfen on brain development and structure.

9902509 Identification of Pathogenicity Mutants of *Colletotrichum graminicola*

Thon, M.R.
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Postdoctoral Fellowship; Grant 99-35303-8191; $90,000; 2 Years

Colletotrichum graminicola is the causal agent of anthracnose leaf blight and anthracnose stalk rot of corn. While this fungus has been recognized as a pathogen of corn in the U.S. since the 1960s, there has recently been an increase in the prevalence of this disease, possibly due to changing agricultural practices. The goal of this project is to identify genes from C. *graminicola* that are important for pathogenicity to corn leaves. A procedure known as insertional mutagenesis will be used to create fungal strains (transformants) that contain randomly disrupted genes. This procedure simultaneously disrupts and tags genes with a short piece of DNA. Transformants will be tested on corn seedlings in order to identify those with a disrupted pathogenicity gene. Since the disrupted genes are tagged, they can be easily isolated for subsequent study of function. The objectives of this project are: 1) perform tests of transformants to identify those with increased or decreased levels of aggressiveness to corn seedlings and 2)characterize the mutants found in step one through classical and molecular genetic analysis in order to identify the gene.

9903351 Bovine IL-12 Receptor Gene Expression

White, A.M.
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Postdoctoral Fellowship; Grant 99-35205-8528; $89,547; 2 Years

Brucellosis, a disease caused by the intracellular pathogen, *Brucella abortus*, affects both humans and cattle. In cattle, it results in late spontaneous abortions and prolonged infertility. This is particularly important when considering dairy production, as aborting cows have decreased milk production. In addition, the loss of calves and the interruption of any breeding program is costly. Humans can contract brucellosis from ingesting unpasteurized milk or from handling an infected cow or aborted fetus. The disease manifests itself in several forms from artritis, nervousness, and uncontrolled shaking to undulant fever and meningitis. In an effort to better understand the immune response to this pathogen and aid in the genetic improvement of cattle, I plan to study the bovine Th1 immune response by analyzing the expression of the interleukin 12 receptor known as IL-12Rb2. The Th1 immune response is particularly important in combating intracellular pathogens such as Brucella, Mycobacteria, and Listeria and is used to control protozoan parasites such as Cryptosporidia and Toxoplasma in some mammals. It has been shown in other mammals that immune response variations among individuals is controlled both by the ability of macrophages to make IL-12 and by the expression of the high affinity IL-12 receptor. I hypothesize that this will be similar with cattle. If true, then this knowledge will help create better vaccine cocktails to protect both animals and humans from these intracellular pathogens.

9901898 The Evolution of Floral Homeotic Gene Expression

Fuerstenberg, S.I.
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Postdoctoral Fellowship; Grant 99-35304-8131; $88,000; 2 Years

The evolutionary innovation of the angiosperm flower sparked a great radiation, establishing angiosperms as the dominant plant group on earth. Yet little is known about flower origins. Recent studies in plant development have established the roles of several genes involved in floral development, providing a new tool for the study of the evolution of gene expression. Gnetales, the sister group to angiosperms, have many characteristics otherwise restricted to the angiosperms, including flower-like reproductive units. This proposal will address the evolution of the flower by determining

the expression patterns of floral homeotic genes in the gnetophyte *Welwitschia*. Degenerate PCR primers will amplify regions of homologous genes, which will be used as probes to isolate full-length clones from genomic and cDNA libraries. Nucleotide and inferred protein sequences will be compared to the sequences of homologous genes to determine changes that have occurred over evolutionary time. *In situ* hybridizations will determine the spatial expression patterns of these genes and establish homology to floral organs. Northern hybridizations on dissected reproductive units and other tissues will determine the relative levels of gene expression. This study addresses the origin of flowers, the evolution of gene expression, and the molecular-developmental basis of morphological change.

9901880 Molecular Cloning of *STUNTED PLANT-1*, a Gene Modulating Cytokinin Response

Schuerman, P.L.
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Postdoctoral Fellowship; Grant 99-35304-8141; $90,000; 2 Years

Plant hormones are chemical compounds produced in plants which direct growth and development. Understanding how hormones achieve their effects in plants provides us with knowledge which can be used to alter how plants grow and develop, and thus improve agriculture. One important class of hormones in plants are the cytokinins. Cytokinins play a role in a multitude of processes, and are known to promote cell division, redirect the distribution of metabolic resources, alter the overall form of the plant, and increase lifespan by inhibiting senescence. However, the mechanisms by which cytokinins achieve these effects are poorly understood. To study the mechanism of cytokinin action, we propose to use the *Arabidopsis thaliana* mutant *stunted plant-1 (stp1).* The mutant has multiple physiological characteristics that are consistent with elevated cytokinin sensitivity. The project will characterize the mutant in three ways: physiologically, genetically, and by cloning the *STP1* gene. By understanding the nature of the mutated gene, it will be possible to reconstruct the normal processes involved in the plant's cytokinin perception and response. This will provide knowledge that will be used to develop a model for the mechanisms by which cytokinins work in plants.

9900944 Landscape Scale Interactions Between Fire Regimes and Ecosystems

Miller, C.L.
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Postdoctoral Fellowship; Grant 99-35101-7828; $88,000; 2 Years

Fires play an important role in many ecosystems by removing accumulated fuels, creating establishment sites for new plants, enhancing wildlife habitat, increasing water yields, and increasing the availability of soil nutrients. Fire suppression during the past century has contributed to the deterioration of many fire-dependent ecosystems and to the increase in uncontrollable wildfires. Although managers are striving to restore the process of fire to fire-dependent ecosystems, most of our understanding of fire lies in its short term effects and does not extend to the large spatial scales at which managers must manage. An understanding of how fires and ecosystems interact over long time frames and at large spatial scales is necessary if we are to sustainably manage fire-dependent ecosystems. The objective of my research is to provide guidance for fire management and planning through an improved understanding of the interactions among topography, vegetation, and fire. I have three specific objectives: (1) improve how fire regimes are described at large spatial scales, (2) develop a computer model to determine how topography and vegetation influence the occurrence, severity, and spatial distribution of fires, and (3) use the model to investigate the long term interactions between fire and vegetation distributions across landscapes. I will develop a computer model for the Selway-Bitterroot Wilderness Area in northern Idaho and western Montana to simulate fire patterns and vegetation dynamics. Historical fire data will be used to test the model and to evaluate new approaches for describing fire regimes. My goal is to assess the long term consequences of fire management activities on vegetation distributions and fire regimes.

9901347 Genetic Diversity and Evolution of a Defense Trait in Maize and Its Wild Relatives

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Postdoctoral Fellowship; Grant 99-35301-8076; $90,000; 2 Years

Crop loss to herbivores and pathogens is a major limitation to agricultural production. Herbivores and pathogens are also a major selective force in natural ecosystems. Although the mechanisms plants have evolved to limit tissue and seed loss to herbivores (defense mechanisms) have been subject to intensive investigation by biochemists, ecologists and evolutionary biologists, relatively little is known about the evolutionary dynamics of defense mechanisms. The main goal of the proposed research is to analyze information contained in DNA sequences to understand evolutionary dynamics of one type of plant defense, protease inhibitors, in maize and its wild relatives. The specific objectives are to determine i) whether defense mechanisms are changing continuously or are maintained over long periods of time, and ii) the temporal and geographical scales at which defense mechanisms evolve. These objectives will be met by isolating and sequencing protease inhibitor genes from 10-20 plants from each of two geographically distinct populations of maize and two closely related wild species. Recently developed statistical techniques will be used to analyze the information contained in the gene sequences.

In addition to providing unique insights into the evolutionary dynamics of plant defense this research will i) identify genetic resources that may be useful in crop improvement, and ii) may provide information that is useful in developing efficient strategies for sampling genetic diversity for defense mechanisms from natural populations of agronomically important plant species.

9900598 Regulation of Metal Uptake in Plants

Connolly, E.L.
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Postdoctoral Fellowship; Grant 99-35100-7762; $90,000; 2 Years

Many metals, like iron and zinc, are essential nutrients, and the ability to accumulate these nutrients from the diet is crucial for human health. Currently, more people suffer from malnutrition due to micronutrient deficiencies than from deficiencies of protein or energy and it is estimated that 2.7 billion people worldwide suffer from iron deficiency (http://www.who.int/nut/malnutrition_ worldwide.htm#ida). In agriculture, the availability of metals in soil plays a major part in determining crop yields. In addition, plants are the main dietary source of these essential metals for much of the world's population. However, metals can also be toxic to both plants and animals when they are present at high levels. Thus, all cells must carefully regulate their metal levels. In particular, the transport of metals into the cell is a primary control point for the regulation of metal levels. The long range goal of these studies is to understand how cells regulate the uptake of metal ions. The central hypothesis of this research is that a novel group of proteins named the ZIP family (for ZRT-like and IRT-like Protein) plays an important role in the uptake of metals by cells. Specifically, I will examine the regulation of two ZIP transporter proteins, IRT1 and ZIP3. The proposed studies will ultimately lead to a better understanding of metal uptake in plants and to the design of plants that are capable of growth on micronutrient deficient soils and that accumulate metals like iron and zinc and thus have a higher nutritional value.

9900852 The Relationship between Species Richness and Exotic Plant Invasion

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Postdoctoral Fellowship; Grant 99-35315-7695; $76,172; 2 Years

Exotic plant invasions are ubiquitous in the modern agricultural landscape. However, the relationship between biological diversity and invasion by exotic plant species has rarely been tested experimentally. This project will examine invasions by exotic plant species into abandoned agricultural fields to address two basic ecological questions: 1) Does the number of species in a community influence the invasion of exotic plant species into that community? and 2) Does invasion by exotic plant species subsequently affect the number of species in a community? Both of these ideas have been proposed in recent ecological literature, and both may result in the commonly observed negative relationship. The relative importance of these causes and effects of exotic plant invasion must be determined to develop practical management strategies for disturbed lands. This project will answer these questions using two analytical approaches. The first will utilize a long term data set on old field succession at the Hutcheson Memorial Forest Center to examine exotic plant invasions and their effects over a 40 year period. This data set is the longest known continuous study on succession, and provides a unique opportunity to study exotic plant invasions through time. The second approach is an experiment that will use an exotic species addition to specifically test hypotheses about the limiting factors and consequences of exotic plant invasions. Information from this study can be directly applied to many agricultural systems such as grazing, forestry, and crop rotations, to aid in the assessment and management of exotic plant species.

9900707 Regulation of the Mobilization of Stored Vitamin A

Gamble, M.V.
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Postdoctoral Fellowship; Grant 99-35200-7612; $90,000; 2 Years

Despite the extreme day-to-day variability in dietary vitamin A intake, relatively constant levels of retinol are maintained in the circulation. All circulating retinol is transported in the blood bound to its specific transport protein, retinol-binding protein (RBP). It is generally accepted that hepatocytes represent the major source of RBP-retinol, and that RBP-retinol release from hepatocytes is responsible for maintaining constant serum RBP-retinol levels. The mechanisms responsible for regulating mobilization of hepatic RBP-retinol stores is not well characterized. However, it is known that RBP secretion by hepatocytes is dependent on retinol availability. Under conditions of vitamin A-deficiency, RBP accumulates in the endoplasmic reticulum (ER) of hepatocytes, and upon addition of retinol, accumulated RBP is rapidly secreted. Since RBP mRNA levels remain fairly constant under most conditions, it is likely that regulation occurs post-translationally. Like RBP, apolipoprotein B100 (apoB) is a secretory protein which is synthesized in hepatocytes in great excess of that which is secreted, and which is dependent upon lipid binding for secretion. RBP secretion may mimic apoB secretion, and in fact, data available to date suggest that they share common pathways and/or chaperone protein interactions. The long-term goals of this proposal are to carry out experiments to enhance our understanding of the

regulation of RBP-retinol secretion by hepatocytes. These studies will be modeled on studies which have explored the regulation of apoB secretion. A greater understanding of retinol-RBP secretion will have a marked impact on our understanding of whole body vitamin A metabolism.

9902859 Novel Strategies for Determining Thermal Destruction of *Mycobacterium paratuberculosis*
Sasahara, K.C.
Cornell University; Department of Food Science; Ithaca, NY 14853
Postdoctoral Fellowship; Grant 99-35201-8039; $87,784; 2 Years

The American dairy industry annually loses $120 million to Johne's disease, an incurable bacterial infection in cattle. The infection is caused by the bacterium *Mycobacterium paratuberculosis*. Infected cattle shed *M. paratuberculosis* into milk and feces, posing a possible health risk to humans. Although pasteurization of raw milk kills most spoilage and pathogenic microorganisms, isolation of *M. paratuberculosis* from humans suffering from Crohn's disease, an incurable inflammatory bowel disease with clinical symptoms similar to Johne's disease, suggests a possible causal relationship. Processing times and temperatures that will enhance destruction of *M. paratuberculosis* in milk remain to be established. Current standard culturing techniques for enumerating viable *M. paratuberculosis* cells will detect the presence of low numbers, but are laborious, time-consuming and may not allow detection of sublethally heat injured, viable but non-culturable cells (VNC). The VNC may represent a significant proportion of cells surviving heat treatment. To better establish whether *M. paratuberculosis* cells are killed by pasteurization, we will use *M. paratuberculosis* strains engineered to produce light when viable to test the efficacy of pasteurization and to develop a strategy for screening for the presence of *M. paratuberculosis* in raw and processed milk. At the conclusion of this project, we will have established specific heat treatment parameters for the destruction of *M. paratuberculosis* and will have developed a rapid detection method to improve dairy herd health management.

9901582 The Function and Evolution of Plant Myb Proteins
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Postdoctoral Fellowship; Grant 99-35301-8102; $90,000; 2 Years

Understanding the molecular mechanisms that control plant metabolism and form is fundamental to the use of genetic engineering to rationally manipulate specific features of crop plants. Because they can alter the expression of multiple genes, transcription factors (proteins that control gene expression) represent excellent targets for genetic manipulation. Myb proteins are members of a diverse family of plant transcription factors, and more than 100 distinct genes encoding Myb transcription factors are present in typical crop plants. Plant Myb proteins are related to a smaller family of animal transcription factors, but the plant and animal Myb proteins exhibit fundamental structural differences. Myb proteins from plants (designated pc-Myb proteins) that exhibit greater structural similarity to the Myb proteins present in animals than to the other Myb proteins in plants were identified recently. The identification of the novel pc-Myb proteins conserved between animals and plants and the existence of the diverse family of plant Myb proteins suggests that the smaller pc-Myb (and animal Myb) family represents an ancient group of proteins while the diverse plant Myb family represents a relatively recent innovation within the plants. The focus of this research will be to determine whether the ancient group of Myb proteins regulate a set of genes that are involved in fundamental cellular processes shared by plants and animals, and to examine whether specific plant Myb proteins regulate functions unique to plants. These results should provide information that may allow the use of some Myb genes in the genetic engineering of crop plants with the consequence significance for agriculture.

9902874 Evalution of Jenseniin G as a Potential Food Preservative
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Postdoctoral Fellowship; Grant 99-35201-8265; $90,000; 2 Years

Bacteriocins, proteins produced by some bacteria which kill other bacteria, offer a defense against some foodborne pathogens. Currently, nisin is the only approved bacteriocin for use in foods in the United States. The development of additional bacteriocins for use as food preservatives is needed in the event organisms develop resistance to nisin or for use in foods in which nisin is ineffective. Jenseniin G, a bacteriocin produced by *Propionibacterium jensenii*, inhibits the outgrowth of the causative agent of botulism and is heat and pH stable. These qualities make jenseniin G attractive as a biological food preservative and this proposal addresses its evaluation. Objectives include increasing the number of organisms which are sensitive to jenseniin G, characterizing the mode of action of jenseniin G, and producing large amounts of jenseniin G. Foodborne pathogens will be subjected to conditions which will weaken the bacteria to determine if the compromised cells become more sensitive to the action of jenseniin G. The mechanism by which jenseniin G kills other bacteria will be determined and compared to that of nisin, a known pore-forming bacteriocin. If the mode of action of jenseniin G is different, then the simultaneous addition of jenseniin G with a pore forming bacteriocin may increase the

efficiency of killing above that achieved by either bacteriocin alone. Large quantities of protein which are difficult to obtain from *Propionibacterium* will be needed if jenseniin G is to be used as a food additive. Three systems will be examined for the over production of jenseniin G.

9902304 Effects of Vertical Infections of *Renibacterium Salmoninarum* on the Salmon Immune Response

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Postdoctoral Fellowship; Grant 99-35204-8587; $90,000; 2 Years

Renibacterium salmoninarum (Rs), causative agent of bacterial kidney disease (BKD), is one of the most important pathogens of wild and cultured salmonid fishes worldwide. The pathogen causes systemic infection that can be transmitted both horizontally (fish to fish), and vertically (parent to progeny). There is no effective way to control the disease using antibiotics or vaccines. The potential for the development of immunological tolerance to Rs due to vertical transmission is a variable that has not been adequately investigated. Salmon fry infected with Rs as unfertilized eggs may be unable to distinguish important protective Rs immunogens from self proteins, leaving them unresponsive to vaccination. The purpose of this research is to determine the effect of vertical transmission of viable Rs cells on the development of the salmon immune response to vaccination and challenge with Rs compared to controls. Specific objectives are to determine if vertical infection affects vaccination efficacy or antibody and macrophage responses, and if the effects are due to a generalized immunosuppression or specific immunological tolerance to Rs antigens. This research will increase knowledge needed to prevent or reduce severity of disease in salmonids by providing information necessary for development of more effective immunization strategies for BKD. The results could also be relevant to other vertically transmitted pathogens in salmon as well as other aquaculture species.

9902973 The Impact of Generalist Predators in Agricultural and Refuge Habitats

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Postdoctoral Fellowship; Grant 99-35302-8626; $90,000; 2 Years

The goal of this project is to improve conservation, and thus biological control effectiveness, of two important types of predators: ground beetles and wolf spiders. These predators, both of which are native to the United States, occur in a wide variety of crop systems and have been found to control pests. Ground beetles and wolf spiders move into agricultural fields from surrounding, less-disturbed habitats that serve as refuges. Thus the effectiveness of these predators as biological control agents depends in part on their densities in surrounding, non-agricultural habitats. The focus of the proposed research is a field experiment where the immigration rate of ground beetles and wolf spiders will be manipulated to establish vegetable plots with different combinations of these predators. Each field plot will be divided into subplots containing 1) cucumbers or squash and no other plants; 2) three different plant species planted as a refuge for predators; and 3) complex plant communities including many plant species planted as a refuge for predators. The resulting impacts of the predator manipulation on other predators, plant-feeding insects, and plant weights will then be measured, to determine the impact of the ground beetles and wolf spiders in both the agricultural and refuge habitats. Conservation of native predators can increase the effectiveness of biological control. Ground beetles and wolf spiders are common in agricultural fields. More effective conservation of these abundant predators can increase their role in pest control, and thus lessen the need for insecticide applications.

NEW INVESTIGATOR AWARDS

New Investigator Awards are intended to enhance the ability of investigators beginning their research careers in obtaining competitive grants from the NRI. New Investigator Awards are reviewed in the program area most appropriate for the type of research proposed; therefore, these nontechnical summaries also appear under the respective program areas.

9900815 Metabolic Genomics: A Novel Technology to Assess Nutrient Status

Vulpe, C.
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New Investigator Award; Grant 99-35200-7578; $230,000; 2 Years

With every meal the body extracts from the diet the nutrients required for its needs. For every nutrient from the abundant, such as proteins and carbohydrates, to the scarce, including vitamins and minerals, complex mechanisms regulate uptake from diet, utilization by the body, and excretion of excess. Disease, inadequate diet or excessive nutrient loss can disrupt this delicate balance and detrimental nutrient deficiency or excess results. Clinical assessment of nutrient status of an individual is complicated by the vast number of required nutrients, the intricate mechanisms for balancing need and excess, and the subtle clinical findings of nutrient deficiency or excess. In the absence of characteristic findings present in severe nutritional disorders, a clinician is faced with a bewildering selection of possible diagnostic tests for a suspected nutritional disorder. Alternative robust methods able to simultaneously assess multiple nutrients, tolerant of the complex interactions between nutrients and yet able to discern specific nutritional disorders, are needed. We propose to develop a novel methodology to assess the nutritional status of an individual by monitoring the metabolic response of the individual to changes in its environment which may provide the means to determine the nutritional state of an individual. We will monitor one critical aspect, changes in gene expression, as an indicator of the metabolic response to nutrient deficiency or excess. Recognizable nutritional response patterns could then be used in clinical assessment to monitor the nutritional status of an individual or to identify a nutritional deficiency or excess.

9900651 Effect of Fish Oil on Hepatic Lipid and Carbohydrate Metabolism in Healthy Human Subjects

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New Investigator Award; Grant 99-35200-8605; $150,000; 3 Years

Large-scale studies have confirmed that the frequency of cardiovascular disease was much lower in Eskimos who consumed traditional foods when compared to populations consuming Western diets. This difference was not explained by the quantity of dietary fat (similar in the Eskimo and Westerners) but by the source of fat (fish versus land animal and plant, respectively). These observations sparked extensive investigations, which determined that the beneficial effects could be attributed to fish oil. It is now recognized that fish oil has a wide range of biological effects on blood fat, platelets, blood clotting, blood pressure and others, with the potential to protect against the risk of cardiovascular disease. More specifically, blood levels of a particular fat called triglyceride, which is associated with cardiovascular disease, can be reduced by 20 to 40 percent. At present, although fish oil has a recognized effect on lowering triglyceride levels, it is not known how this effect operates. The consequences of this effect on liver glucose metabolism are also unknown. We propose that: 1) the reduction of triglyceride by fish oil is due to a reduction of the conversion of carbohydrate to fat in the liver, and 2) fish oil may increase glucose synthesis and production by the liver. To find out whether this proposal is true, we will measure the synthesis of fat and glucose in healthy subjects when they are receiving either fish oil or a placebo in their diet. This study will provide important information for understanding the mechanism of fish oil actions.

9901894 Functional Analysis of a Kinetochore-Associated Kinesin-Related Protein

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New Investigator Award; Grant 99-35304-8142; $150,000; 2 Years

Cell division is an essential process by which living organisms grow, develop, and reproduce. In flowering plants, cell division is the only way that certain organs develop to their mature shapes, because plant cells normally do not move around. Cell division must occur with high fidelity so that the genetic content gets maintained in a constant level among cells within a species. A fibrous protein network of microtubules is largely responsible for cell division to succeed. During cell division, microtubules organize into a dynamic array called the spindle apparatus that conducts the even segregation of genetic material-bearing chromosomes. Each replicated chromosome is attached to the spindle apparatus at its sibling kinetochores so that the sister chromatids will segregate equally into two daughter cells. In order to do so, microtubules undergo dynamic reorganization, which is believed to rely on a group of enzymes named motor proteins. They are kinesin and its related proteins and dyneins. To elucidate the mechanisms of plant cell division, we need to characterize these motor proteins that play crucial roles in this fundamentally important process. This proposal is aimed at characterizing one of the kinesinrelated proteins that plays a role in regulating the activity of chromosomes. We are going to determine the amino acid sequence of this protein in model plants rice and *Arabidopsis* by cloning the genes. In order to understand its function, we will also isolate this kinesin-related protein so that we can dissect its activities *in vitro.* Finally, we want to understand how this protein functions during cell division by taking cell biological approach. Information about the activities of the kinesin-related proteins will help us understand how plant cells divide so that ultimately it will help us understand the essence of plant growth and development. Such knowledge will be very useful for improving crop yields in the near future.

9901679 A Novel Integrated Process for Pilot-Scale Production of Xanthan Gum

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New Investigator Award/Strengthening Award; Grant 99-35503-8664; $140,000; 3 Years

Xanthan gum, the most important microbial polysaccharide, is widely used as a suspending and thickening agent in the food, chemical, and pharmaceutical industries. However, current industrial production using conventional stirred-tank fermentors is energy-intensive and costly. This is mainly because the high viscosity causes agitation and aeration to be difficult and limits the final yield and productivity. The problem encapsulates challenges in both fermentor design and operation, the key areas in fermentation process development. Our previous work has shown that, by using a novel centrifugal, packed-bed bioreactor (CPBR), xanthan productivity can be elevated significantly. Moreover, incorporation of membrane ultrafiltration (UF) to concentrate fermentation broth before alcohol precipitation can save up to 80% of energy used in downstream recovery. In this proposal, we consider not only the engineering challenges inherent in many scale-up operations but also the quality and functionality of the product produced from the new process, mainly the rheological characteristics of xanthan polymers. The overall project goals are to: (1) design and optimize the CPBR for pilot-scale xanthan gum production; (2) engineer the performance of UF process to further improve xanthan gum recovery; and (3) evaluate and control the quality and functionality of xanthan polymers produced from this process. Not only will this research project improve our knowledge on the scale-up operation of a novel integrated fermentation process for xanthan gum production, but it will also provide insights on critical issues associated with cell metabolism and product formation kinetics.

9902225 Bovine Somatotropin-Mediated Effects to Increase Embryonic Survival in Cattle

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New Investigator Award; Grant 99-35203-7676; $140,000; 2 Years

A major cause of lowered calf crop and increased calving intervals in dairy operations is suboptimal embryo survival. Bovine somatotropin (bST) increases the rate of early embryonic development in cattle. Goals of this project are to examine the physiological windows and the mechanisms by which bST affects fertility responses in lactating dairy cattle. Our hypothesis is that pregnancy losses often occur because of the failure of the uterus to provide an environment that is conducive to optimal embryonic development and that a portion of these embryos can be rescued by supplemental bST administration to increase availability of insulin-like growth factors (IGFs) that are essential for early embryogenesis. The first objective is to evaluate the stages of pregnancy when embryonic development and survival are most affected by exogenous bST. The second objective is to characterize bST-induced changes in oviductal, uterine and embryonic synthesis of IGFs and their binding proteins (IGFBPs) and to examine whether these biosynthetic activities can be correlated with IGF and IGFBP contents of oviductal and uterine fluids. The third objective is to determine how bST stimulates embryonic development using an *in vitro* model. The fourth objective is to examine the effect of supplemental bST on uterine synthesis of prostaglandin F2 alpha which causes luteal regression in cattle. Knowledge gleaned from this research will provide new strategies to improve embryo survival and pregnancy rates in lactating dairy cows in which fertility is reduced due to high metabolic demands of high milk production.

9903277 Production of All-Female Stocks of Pacific White Shrimp

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New Investigator Award/Strengthening Award; Grant 99-35206-8258; $130,103; 2 Years

The objective of this project is to establish methods to produce all-female populations of Pacific white shrimp (*Litopenaeus vannamei*) to increase productivity of U.S. shrimp farms. Female white shrimp are 10-20% larger than males at harvest. Our long-range goals are to develop economically viable methods of producing all-female shrimp for U.S. shrimp farmers. We propose to produce all-female shrimp populations by: 1) application of hormones in the water or feed; 2) manipulation of environmental conditions that may affect sex ratios; and 3)determination of family differences in sex ratios and selective breeding for skewed sex ratios. These approaches may allow for the commercial production of all-female shrimp populations as is typically done in salmonid farming.

The U.S. has an annual trade deficit in shrimp products in excess of $2.5 billion. With commercial shrimp fisheries at maximum sustainable yield, increased domestic production of shrimp will likely come from aquaculture. Currently, shrimp farmers rely on outdoor, earthen ponds situated along the coast. These farms are marginally profitable and there is little room for expansion of the industry. Future expansion of the U.S. shrimp farming industry may be dependent on biosecure production systems built away from the coast with little negative environmental impacts. Production of larger, all-female shrimp will increase the profitability of current shrimp farms, as well as the emerging inland, biosecure production systems. Increasing profits and productivity in these more environmentally friendly systems may allow for future expansion of the U.S. shrimp farming industry to all areas of the country.

9901133 Assessing the Vulnerability of Farmstead and Rural Domestic Wells to Agrichemical Contamination
Ray, C.
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New Investigator Award; Grant 99-35102-8551; $88,000; 2 Years

The goal of this project is to assess the vulnerability of farmstead and rural domestic wells to pesticide and nitrate contamination. Rural domestic wells do not come under any regulatory compliance for water quality testing. Periodic testing of individual wells is not feasible due to cost and logistics. However, the assessment of vulnerability of these wells, using data from some large-scale studies in the mid-continental United States is proposed. Artificial Neural Networks (NN) will be used for vulnerability assessment utilizing the occurrence data of nitrate and pesticides, well site land use information, well construction details, and other climatic, hydrologic and geologic data. The NNs are multi-input/multi-output models that have the capability to account for complex interaction among the input parameters that contribute to domestic well contamination. The NNs have been successfully used in other hydrologic applications such as rainfall-runoff modeling. As more pesticide and nitrate occurrence data become available along with site information, the NN model can be retrained with the new data and the model can be tested against sites with such data. Using a trained and tested NN model, contamination potential for new sites with adequate site and hydrologic data can be predicted in the same region. The identified high-risk sites can be taken up for confirmatory sampling by the health or regulating agencies. The contaminated wells thus can be identified for remedial measures and the well user can take other measures to prevent future contamination. Potential cost savings can be realized by testing only the most vulnerable wells.

9901234 Social and Economic Influences on Rural Small Business Competitive Strategy
Miller, N.J.
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New Investigator Award; Grant 99-35401-7767; $120,000; 2 Years

Recognizing the important role small businesses play in strengthening the economies of rural communities, this project focuses on the strategies used by rural small business owners /managers to enhance their competitiveness in regional, national, and global markets. The net benefits of small business success are distributed community-wide. In addition to the provision of goods, services, and employment opportunities, small businesses contribute leadership and support to a broad array of community betterment efforts. The theoretical framework suggests that the social characteristics of communities can affect the success of small businesses. The research proposed here will examine the reciprocal and multifaceted relationship between small businesses and rural communities, specifically focusing on the embeddedness of small businesses in their community, their strategies for success, and their perceived and financial success. Features of the community, business, and the personal characteristics of the owner/ manager will also be examined. This investigation provides a new direction by measuring influences on the competitive strategies of rural small businesses and the relationships of these elements to business success. The unit of analysis will be small business owner/managers operating in two samples of Component Economic Areas (CEA), a concept developed by the Department of Commerce. These are multi-county units characterized by internal economic linkages. Small businesses in rural counties in CEAs with a metropolis and those in rural counties in non-metropolitan CEAs will be compared. This project will address a weakness in the literature, as well as provide insight into small businesses as a critical area of rural community development.

9901591 Future of Indian Grain Supply and Demand: Implications for U.S. Grain Exports
Mohanty, S.
Iowa State University; Department of Economics; Ames, IA 50011
New Investigator Award; Grant 99-35400-7697; $105,000; 2 Years

With its large and growing population, the Indian market for U.S. grain exports is limited only by policies that restricts imports and by relatively low level of incomes. In recent years, however, the government of India has implemented economic reforms that have stimulated economic growth and begun the process of liberalizing agricultural markets. Such changes open the possibility of rapid growth in import demand. The purpose of this project is to develop a theoretically consistent framework incorporating the effects of urbanization on food demand, the growing importance of livestock production and the associated demand for feed grains, regional variations in grain production within India, and the influence of domestic and trade policy interventions on production, consumption and trade. The model that will be constructed to analyze the prospects for U.S. grain exports to this important market includes an acreage allocation model to measure regional grain production, separate food demand models for rural and urban households specified as dynamic AIDS models, a feed demand model, and a model of livestock production. These models are linked to the world market through world prices, export and imports. Data for estimating the parameters of the model will be collected from secondary sources as well as through household surveys carried out by collaborators in India. The model will be used to analyze several scenarios concerning the likely evolution of Indian grain imports in light of increased urbanization, the production potential for Indian producers, income growth and continued policy reforms.

9901772 Structure and Function of Non-Symbiotic Plant Hemoglobins

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New Investigator Award; Grant 99-35306-7833; $210,000; 3 Years

We will conduct a study of the structure and function of a new, ubiquitous class of plant hemoglobins which are linked to the anaerobic response in plants and a possible new mechanism for respiration under low oxygen concentrations. Every year, these conditions are brought on by flooding which results in the loss of millions of dollars in agricultural products annually in the United States. The effects of flooding on crop yields can be ascribed to hypoxia resulting from the inability of oxygen to easily diffuse through water or waterlogged soil. The marked differences in survival under hypoxic conditions among different plant species suggest much room for improving the yields of most crops through breeding or recombinant techniques. However, we must first understand the biochemical factors which determine the ability of plants to survive hypoxic conditions.

This new class of hemoglobins (the nonsymbiotic hemoglobins or nsHbs) is distinct from the more familiar symbiotic plant hemoglobins involved in nitrogen fixation in some legumes. Because of their unusually large affinities for oxygen and low intracellular concentrations, it is unlikely that nsHbs are involved in oxygen transport. Instead, it has been suggested that they are involved in a new metabolic mechanism for energy production under low oxygen conditions which allows plant survival without the harmful buildup of fermentation products. The long term goal of our investigation of these proteins is to exploit their function for engineering the survival of crops in oxygen limiting environments.

9900622 Sulfur Stress Signal Transduction

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New Investigator Award; Grant 99-35100-7579; $200,000; 3 Years

An adequate supply of nutrients is required for maximum crop productivity and quality. However, agricultural lands are often deficient in one or more of the essential nutrients. Plants can sense their nutrient status and acclimate to nutrient changes in nutrient availability, but plant growth and quality are often diminished. We are studying how photosynthetic organisms sense and acclimate to sulfur deficient conditions. Sulfur is an essential nutrient and is a component of proteins, lipids, carbohydrates, electron transport chains and numerous intermediate metabolites. Plants preferentially import sulfur as the sulfate ion. However, sulfate supplies in the soil can be low even when other forms of sulfur are plentiful. To understand how photosynthetic organisms sense and respond to sulfate deficient conditions, we have been studying the unicellular green alga *Chlamydomonas reinhardtii*. This organism is related to vascular plants but is easier to work with in the laboratory. It also has some enzymes, absent in vascular plants, that enable it to access alternative forms of sulfur when sulfate is not available. We have isolated, characterized and complemented mutants of *Chlamydomonas* that do not acclimate to sulfate deficient conditions. These mutants have genes (already identified) that enable *Chlamydomonas* to sense sulfate levels and/or control its responses to sulfate deficiency. These genes will enable us to understand these processes at the biochemical level. This research may provide us with tools for early detection of sulfur deficient conditions and enable us to understand how plant productivity is limited by sulfur deficiency.

9903271 Skeletal Muscle Growth by Regulation of Fibroblast Growth Factor Receptor 1 Gene Expression

DiMario, J.X.
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New Investigator Award; Grant 99-35206-7987; $182,000; 2 Years

Consumption of chicken in the United States accounts for a significant amount of total meat. From 1980 to 1997, chicken consumption per capita increased by 56.6%. In comparison, total meat consumption over the same period increased only 6.9%. The research funded by this grant focuses on a critical factor in the development and growth of the chicken musculoskeletal system. Skeletal muscle cells grow in response to the presence of growth factor proteins – particularly fibroblast growth factors (FGFs). These factors bind to cell surface receptors (FGFRs) which transmit signals into the cell for continued cell proliferation. Therefore, both the growth factor and its receptor are necessary for muscle cell growth. Conversely, a decrease in growth factor and/or its receptor is required for muscle cell differentiation and formation of normal muscle tissue. Our research has shown that the receptor for fibroblast growth factor is down-regulated during chicken skeletal muscle differentiation. To understand the mechanism of this important process in myogenesis, we have isolated the FGFR1 gene and the associated DNA that regulates its expression. The goal of our research is to understand how this gene is regulated. This will be done by identifying and characterizing the DNA elements that are involved in FGFR1 gene expression in both proliferating and differentiating muscle cells. Also, we will determine which protein factors interact with these DNA elements to regulate FGFR1 gene expression. This research will provide important information concerning how muscle cells grow to form normal muscle tissue as well as contribute to our basic understanding of cell proliferation and differentiation.

9902282 Influence of Paired Testicular Diameter and Age on Sperm Production in the Boar

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New Investigator Award; Grant 99-35203-7646; $29,692; 1 Year

Previous studies in the boar have established that testicular weight correlates well with daily sperm production, and that testis size is a highly heritable trait. The boar testes are tightly positioned so that it is impossible to obtain circumferential measurement. Consequently, unlike the bull and ram, use of testis size when selecting animals as breeding prospects does not occur in swine. Real-time ultrasonography (RTU) offers science a non-invasive technique for visualizing and measuring tissues in the live animal, including the testes. Application of this existing technology to the boar would bridge the gap between science and current swine production practices by providing the industry with the means to select for potential high sperm- producing boars for breeding programs based on testis size. Experiments in my laboratory have found that RTU is an easy and repeatable technique for measuring testicular diameter. The proposed experiments will ascertain the feasibility and accuracy of RTU testis measurement in relation to sperm production and boar age. The study will use proven, hybrid boars standing at commercial US studs. Boars will be grouped by age, with RTU measurements of both left and right testis diameters obtained. Paired testis diameters will then be compared to a total sperm number composite which will be calculated from the boar's four previous semen collections. If results are favorable, the impact of this study on the swine industry is anticipated to be immediate and far reaching in how boars will be selected for AI breeding programs.

9902637 Regulation by 5-Hydroxytryptamine of Midgut Ion Transport in Larval *Aedes*

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New Investigator Award; Grant 99-35302-8371; $120,000; 2 Years

Very little is known about midgut ion transport mechanisms in larval mosquitoes. The PI has found that the anterior and posterior stomach regions of larval *Aedes* midgut possess distinct ion transport mechanisms that are regulated hormonally by 5-hydroxytryptamine (5-HT). The objectives of the proposed research are to identify the principal active transport processes of the anterior and posterior stomach, determine the ion transport processes stimulated by 5-HT in each region, and determine the physiological significance of coordination of midgut and Malpighian tubule function by 5-HT circulating in hemolymph. These goals will be met by determining, 1) the direction and magnitude of net flux of fluid and of each of the ions H+, Na+, K+, and Cl; 2) which ions are transported actively and which passively; 3) the effects of acute and chronic changes in ambient salinity and feeding status on fluid and ion ingestion rates, hemolymph 5-HT concentrations, hemolymph ionic concentrations and total body water in feeding and food-deprived larvae, and 4) the effects of inhibition of 5-HT re-uptake on each of these parameters. This basic research will greatly increase our currently very limited understanding of the regulation and function of the larval mosquito midgut, and thus contribute to the long-range development of control strategies for this important disease vector.

9901886 Regulation of F-Actin During Last Epidermal Development in *Arabidopsis*

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New Investigator Award; Grant 99-35304-8525; $216,000; 3 Years

The growth of plant cells depends on inputs from the environment such as temperature, humidity, and light. Growth also depends on intrinsic signals such as nutrient availability and hormone signals. The question of how plant cells translate these diverse types of signals into a coordinated growth response is an important question in plant biology. Although much progress has been made in identifying many of the genes that perceive growth signals, little is known about how these signals are transmitted to downstream components such as the cytoskeleton to control cell growth. The cytoskeleton consists of filamentous arrays that regulate both the delivery of construction materials to the cell wall and the direction in which the cell expands. For example, the actin cytoskeleton forms a 3-dimensional network of "tracks" on which vesicles containing construction materials can travel. The actin tracks are very dynamic, and the arrangement of filaments can change in response to hormone signals, pathogen attack, and light. A mechanistic understanding of how actin organization is controlled is essential to understand how plant cells grow and improve our ability to genetically engineer complex traits. An interdisciplinary approach using *Arabidopsis* epidermal development has been adopted to identify genes that regulate actin organization and growth. This proposal is centered on understanding the function of a transmembrane protein that is hypothesized to regulate cytoskeletal organization during cell elongation. It is anticipated that a careful examination of the *F-ACTIN ALTERED* gene will lead to the identification of additional players in the cytoskeletal growth control pathway.

9902683 *Wolbachia* Infections in Mosquitoes as an Applied Tool for Modifying Natural Populations

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New Investigator Award; Grant 99-35302-7988; $125,000; 2 Years

Mosquitoes are important pests of livestock, causing significant economic impact from biting and blood feeding that can result in annoyance, immune and toxic reactions in the host, and the transmission of disease. The economic repercussions of these attacks include weight gain reductions, lower milk and egg production, reduced fertility, and death. At present, the control of mosquitoes relies primarily on chemical and biological insecticides. However, the development of insecticidal resistance in mosquitoes combined with an increased difficulty in developing and releasing new larvicide and adulticide compounds have generated a growing interest in alternative strategies to complement current control programs. Intracellular bacteria of the genus *Wolbachia* have been suggested as a tool for modifying populations of mosquitoes and other economically important insect pests. Proposed strategies include the use of *Wolbachia* for both population reduction and population replacement. Both of these strategies are based on the ability of *Wolbachia* to induce cytoplasmic incompatibility, whereby the *Wolbachia* infection renders male mosquitoes impotent. The primary goals of this project will include evaluating the potential of *Wolbachia* to modify populations of the recently imported *Aedes albopictus* (Asian tiger mosquito) and developing techniques permitting the application of these strategies. These studies will also provide useful information for the design of similar strategies in additional insect pests of both livestock and plants.

9902709 Polydnavirus-based Transformation of Insect Cells

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USDA Agricultural Research Service; Insect Biocontrol Laboratory; Beltsville, MD 20705
New Investigator Award; Grant 99-35302-8522; $120,000; 2 Years

Polydnaviruses, unusual DNA insect viruses found in some parasitic wasps, are considered non-traditional viruses because they do not replicate outside of specific cells within the female reproductive tract. It was recently observed that polydnavirus DNA from the parasitic wasp *Glyptapanteles indiensis* could integrate itself into chromosomal DNA of cells derived from the natural host, gypsy moth, and was stably maintained. The phenomenon of integration makes this polydnavirus practical for development as a virus-based tool for transformation of insect cells. The goal for this work is to develop a new system for transformation of insect cells through (1) characterization of the *G. indiensis* polydnavirus (GIPDV) and the genes coded within the integrating DNA of its genome; and (2) analysis of *G. indiensis* polydnavirus integration by characterization of integration sites and the viral segments that integrate in gypsy moth cells under laboratory conditions. This will form the necessary groundwork for future development of a GiPDV-based vector for transformation of and gene expression in insect cells. The proposed research has importance to agriculture as a potential new and alternative insect cell transformation system, as part of long-range reduction in use of chemical pesticides by biological suppression and control of insect pests, and as groundwork for long-term transgenic insect strategies for biologically-based pest control efforts.

9902423 Foaming of Wood-Fiber/Thermoplastic Composites

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New Investigator/Strengthening Award; Grant 99-35103-8601; $90,000; 2 Years

Wood-fiber/plastic composites make use of cellulosic fibers as fillers and/or reinforcing agents in the plastic matrix. These composites have a lower material cost and a higher stiffness than neat plastics. Although these composites have been commercialized, their potential for use in many industrial applications has been limited because of their brittleness, lower impact resistance, and higher density compared to neat plastics. The potential range of uses for these materials in innovative applications would be expanded if these shortcomings could be overcome. Recently, the concept of creating microcellular foamed structures in the composites as a means to resolve these shortcomings has successfully been demonstrated by the PI. Microcells have been developed in the composites through a batch foaming process by using carbon dioxide as a foaming agent. Because of the generated microvoids, the impact strength, brittleness, and density of the composites were dramatically improved. These enhancements were achieved without significantly compromising other mechanical properties. Despite these promising results, the batch foaming process used to generate cellular foamed structures in the composites cannot now be implemented in the industrial production of foams because it is not cost-effective. A procedure for continuous manufacture of foamed wood-fiber/plastic composites is required for the expanding commercialization of these products and is the focus of this study. The objective of the proposed investigation is to develop the scientific and engineering basis needed to manufacture wood-fiber/plastic composite foams using a continuous extrusion process. Positive results from this study are expected to lead to a new class of materials with unique properties and characteristics. Improving

the shortcomings of wood-fiber/plastic composites will extend their utilization in a large number of innovative applications and increase the value-added utilization of wood.

9902068 Infectious Recombination System for Porcine Reproductive and Respiratory Syndrome Virus
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New Investigator Award; Grant 99-35204-8185; $183,938; 2 Years

Porcine reproductive and respiratory syndrome virus (PRRSV) causes great economic losses to the pork industry through its ability to produce respiratory and severe reproductive disease in swine. However, little is known about the mechanisms which PRRSV uses to produce these effects. The overall goal of this study is to define a new genetic system to investigate these mechanisms. Former research has developed two ways to genetically manipulate viruses to determine genomic regions of virulence and to study viral pathogenesis, but problems have been encountered with both of these methods. In the proposed study, we will attempt to blend these two methods by preparing DNA clones of overlapping segments of the PRRSV RNA genome, producing RNA copies of the cloned DNA and introducing these RNA copies to host cells. We will then investigate the possibility that new whole virus, which is indistinguishable from natural PRRSV, will be derived by a process called viral recombination. If successful, this proposal will result in a novel and fundamental tool with which to analyze individual properties of the PRRSV genome and may open up avenues of investigation for many other viral pathogens of animals.

9902047 Non-Antimicrobial Action of Macrolides: Role in Modulation of the Inflammatory Response
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New Investigator Award; Grant 99-35204-8411; $87,000; 1 Year

Bovine respiratory disease (BRD) impacts all areas of the cattle industry with cost estimates ranging from 600 - 800 million dollars annually. Improved outcomes are reported by employing effective management, vaccination and antibiotic strategies. Disease remains a major concern, since stress and microbial pathogens play synergistic roles in disease. Treatment is aimed at reducing the growth of bacterial organisms, and controlling the host inflammatory response. Controlling inflammation is key to reducing losses associated with BRD. Several approved drugs modulate the inflammatory response within the lung in addition to killing bacteria.

The proposed research will examine the cellular mechanisms that antibiotic agents employ in the modulation lung inflammation. Specifically, macrolides modulate inflammation by cellular mechanisms involving cAMP and protein kinases. One macrolide, tilmicosin (Mictotill9), modulates inflammation via inducing inflammatory cell death, while minimizing damage to the lung. We will define these mechanisms in the absence of infection by determining the responses of cells isolated from the lung and peripheral blood of animals treated with macrolide antibiotics. We will characterize the responses of these cells to stimuli by defining intracellular signaling, cell mediator production, and functional changes in inflammatory cells. More complete understanding of the inflammatory response will provide needed information to allow development of therapies aimed at more precisely controlling inflammation, and reducing the use of drugs in food animals.

9901501 Plant Polypyrimidine Binding Proteins as Chromatin Derepressors
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New Investigator Award/Strengthening Award; Grant 99-35301-8023; $118,350; 2 Years

Plant and animal cells produce a form of histone H3 protein that assists the maintenance of chromatin structure in differentiated cells. Genes for such histone H3 variants in plants are constitutively expressed despite the presence of typical cell cycle control promoter elements. Phylogenetic gene analysis suggests that polypyrimidine (PPY) sequence elements are required for the constitutive expression. They are located among the promoter elements, in the untranslated regions (UTRs) and in the introns. We hypothesize that they bind proteins similar to the *Drosophila* GAGA factors, which cause chromatin derepression by preventing nucleosome assembly. In *Arabidopsis*, intron insertion with these elements caused strong increase in reporter gene expression and loss of the cell cycle control of a histone promoter. An alfalfa histone H3 promoter, which contains no intron in the 5' UTR but instead similar PPY motifs, conferred high, constitutive reporter gene expression . Gel shift analyses detected specific interactions between plant nuclear proteins and PPY motifs in the 5' untranslated and intragenic regions of the alfalfa gene. This project has the specific aim to confirm whether PPY elements cause high, constitutive gene expression in transgenic plants. Secondly, PPY-binding proteins will be purified from plants, sequenced and related cDNA clones will be isolated. Transgene silencing by heterochromatin is a major problem in plant biotechnology. The use of PPY sequences as natural gene de-repressors in the promoters and other parts of plant genes will be an invaluable tool to prevent gene inactivation and to maintain high expression of a desired gene throughout plant generations.

9902821 Detection of Viable Enterohemorrhagic *Escherichia coli* using Polymerase Chain Reaction and RNA-based Polymerase Chain Reaction

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New Investigator Award; Grant 99-35201-8125; $111,000; 2 Years

Development of strategies to ensure the safety of food is critical for sustaining U.S. agriculture. Recently developed detection methods based on DNA amplification, termed polymerase chain reaction (PCR), offer a high degree of speed, sensitivity and specificity for pathogen screening in foods. Positive results, however, must still be confirmed by time-consuming conventional methods as PCR does not differentiate viable from nonviable cells. Methodologies will be developed to rapidly detect viable enterohemorrhagic *Escherichia coli* (EHEC). A new DNA-based detection method, quantitative competitive PCR (QC-PCR), and an RNA-based detection method, RT-PCR of mRNA, will be developed. The *slt-I* and *slt-II* toxin genes will be used as targets. For QC-PCR, a segment of competing DNA similar to the target DNA will be created. A known constant amount of competing DNA is then added to dilutions of extracted target DNA followed by PCR amplification. The amount of target DNA is quantified by a generated standard curve. QC-PCR at two points during enrichment will be used to determine viability. For RT-PCR, mRNA , which is a known indicator of cell viability, will be used. RT-PCR amplification of *slt* mRNA will be used after a brief enrichment to determine viability. The sensitivity of both methods with respect to viable and injured cells will be evaluated. The methods will be applied to artificially contaminated ground beef samples. The development of nucleic acid based methodologies to detect viable EHEC will be applicable to other foodborne pathogens and will provide a more rapid option for monitoring food safety.

9901742 Genetics and Biochemistry of Wheat Grain Hardness Genes

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New Investigator Award/Strengthening Award; Grant 99-35301-7764; $120,000; 2 Years

Grain hardness in wheat is a primary factor of wheat flour quality. Grain texture or hardness is a function of the physical texture of the endosperm, and influences such quality parameters as milling energy, particle size, starch damage, baking quality, and water absorption. These quality parameters in turn, strongly impact the commercial usage and value of wheat. Hard wheat flour is used primarily for bread, while soft flour is used primarily in cookies, cakes, and pastries. A single locus, *Hardness (Ha),* controls the difference between hard and soft wheat. This gene difference is marked by soft *(Ha)* or hard (*ha*) textured endosperm and large (*Ha*) or small (*ha*) amounts of the marker protein friabilin on the surface of starch granules. Sequence or transcriptional alterations in the two genes that encode friabilin, *puroindoline a* and *b (pinA* and *pinB,* respectively), are inseparably linked to the wheat *Hardness* locus in wheat that is hard textured. The unbroken linkage between putative mutations in these two genes and hard textured endosperm suggests that they together comprise the *Hardness* gene. Additionally, the putative mutation in *pinB,* a simple glycine to serine change is common in hard wheats that are softer than those with the *pinA* alteration, a null mutation. The data suggest that the *puroindoline* genes act together, to effect softness. The experiments proposed are directed at current shortcomings in our knowledge of endosperm grain texture and the project aims to conclusively identify the genes controlling endosperm texture in wheat. Specifically, the objectives of this project are to conclusively identify the *pinA* and *pinB* genes as functional components of the *hardness* gene using transgenic plant technology. The second objective will quantify flour quality difference between hard wheats containing an alteration in *pinB* versus those devoid of the PINA protein.

9902727 Mechanisms and Dynamics of Group Foraging in the Pine Sawfly *Neodiprion lecontei*

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New Investigator Award; Grant 99-35204-8229; $129,810; 2 Years

The red-headed pine sawfly is one of the most destructive defoliators of hard pines in eastern North America, including several ornamental and pulpwood pine species. Unlike many defoliating pest insects, this species feeds in close-knit groups (colonies), and grouping is of great importance to its pest status. Defoliation rate and destructiveness increases disproportionately with group size as a result of greater feeding efficiency and decreased predation. It is therefore of great interest to understand how these insects group and stay together as they feed, and to what extent groups merge to form super colonies. Preliminary studies suggest colonies stay together through the use of chemical markers, as is true of some moth defoliators. The goals of this project are to (1) determine through behavioral experiments exactly how these sawflies communicate to stay grouped as they move among feeding sites, specifically looking at the role of chemical trails; (2) track colony movements to document foraging periodicity and determine the extent of group splitting and refusion; and (3) determine with genetic markers the extent to which super colonies form under natural conditions. Since defoliation potential is dependent on group size in this species, a better understanding of mechanisms underlying group formation will improve management and control efforts based on the behavior of the insect. Learning how these insects group, for example, is the first step toward figuring out a way to disrupt colonies, perhaps by interfering with their chemical trails.

9901428 Mixed Culture Transformation of Anaerobic Cellulolytic Bacteria

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New Investigator Award; Grant 99-35504-7830; $100,000; 1.5 Years

Cellulose waste or biomass is a major by-product of agriculture. Cellulosic biomass can potentially be converted to ethanol or other desired products in one step using bacteria able to produce the cellulase enzymes necessary to break down cellulose while simultaneously fermenting the resultant sugars. These bacteria grow without oxygen (anaerobically) at high temperature. Economic studies offer strong support for the potential of such "consolidated bioprocessing," and several lines of evidence support the kinetic and bioenergetic feasibility of this concept. One attractive strategy for developing organisms capable of consolidated bioprocessing is to genetically engineer the end product metabolism of naturally cellulolytic anaerobic bacteria such as *Clostridium thermocellum*. The major impediment to pursuing this strategy is the absence, in spite of considerable effort, of a gene transfer system for *C. thermocellum* or similar cellulolytic anaerobes. Both the literature and preliminary results reported herein support the efficacy of screening many strains from the environment to find one or more actively cellulolytic strains amenable to genetic manipulation. We propose to pursue this by electrotransformation of cellulolytic enrichment cultures followed by isolation and characterization of presumptive transformants, which differs from the conventional approach of isolation followed by transformation. The objectives of the project are to develop a genetic system for one or more actively cellulolytic bacteria as well as to demonstrate and evaluate the mixed culture transformation approach. Successful completion of the project will provide transformable potential type strains for subsequent studies of both applied and fundamental significance.

9901082 The Effect of Harvester Ant Nests on Nitrogen Cycling in Arid Rangeland Soils

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New Investigator/Strengthening Award; Grant 99-35101-7834; $180,000; 3 Years

The goal of the proposed research is to estimate the effects of a common, soil-dwelling animal species, the harvester ant (*Pogonomyrmex barbatus*), on nitrogen cycling in arid rangeland soils. Often viewed as pests because of their seed eating habits and venomous sting, preliminary data suggest that harvester ants play a significant role in nutrient cycling. Ants concentrate organic debris in the vicinity of the nest, thereby creating islands rich in organic matter and mineral soil nutrients within the landscape. On the study site, harvester ant nests occupy only about 1% of the surface area of the landscape but we estimate that ant nest soils contain 9 - 14% of the total available soil nitrogen on the plot. We hypothesize that ant nests alter major N transformations and play an important role in controlling rangeland soil fertility. The influence of harvester ants on nutrient cycling at large spatial scales depends on the density, age and size of nests. The proposed research will combine information about how ants affect soil nutrient levels and nutrient cycling with information about ant population biology to estimate the effects of harvester ant populations on nitrogen cycling at large spatial scales.

9902758 Transmission of *Listeria monocytogenes* in Food Systems

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New Investigator Award; Grant 99-35201-8074; $192,000; 3 Years

Listeria monocytogenes is a bacterium capable of causing a serious foodborne disease ("listeriosis") in humans. A 1998-9 listeriosis outbreak in the US affected more than 100 people and killed at least 20 people. An estimated 1,700 cases of human listeriosis occur annually in the U.S. About 20-40% of people infected with this bacterium die. Only a few, genetically distinctive *L. monocytogenes* subtypes appear to be responsible for most listeriosis cases, suggesting that not all subtypes are equally capable of causing human foodborne disease. *L. monocytogenes* is commonly present in the environment; thus preventing contamination of food products with this organism is challenging. In fact, most food recalls due to bacterial contamination are a consequence of the presence of *L .monocytogenes*. Current regulations require the recall of any ready-to-eat food containing more than one *L. monocytogenes* cell/25 g. Therefore, *L. monocytogenes* contamination presents a serious economic concern to food processors. This project is designed to better understand the characteristics of different *L. monocytogenes* subtypes and to gain a better understanding of which specific *L. monocytogenes* subtypes cause human disease and how these types differ from those that cannot cause human disease. We will comprehensively characterize *L. monocytogenes* isolates from humans, animals and foods using both DNA fingerprinting methods and methods for evaluating a strain's ability to cause disease. This work will ultimately contribute to the development of science-based food safety regulations that economically meet public health needs.

9902756 Specific Detection and Typing of *Vibrio parahaemolyticus* serotype O3:K6

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New Investigator Award; Grant 99-35201-8075; $110,065; 2 Years

Vibrio parahaemolyticus is a bacterium that is naturally found in marine waters. Shellfish, which obtain food by filter feeding, easily concentrate such bacteria to high levels. Human infection with *V. parahaemolyticus* is most frequently associated with the consumption of seafood, primarily raw or improperly cooked shellfish. While most strains of *V. parahaemolyticus* are not pathogenic to humans, some strains cause gastroenteritis. Of three U.S. outbreaks of foodborne disease linked to consumption of raw oysters in 1997 and 1998, two have been attributed to a unique strain of *V. parahaemolyticus* (the O3:K6 serotype). This *V. parahaemolyticus* strain may have an enhanced ability to cause disease. Currently, identification of the O3:K6 serotype requires extensive biochemical and genetic tests, which are both time-consuming and expensive. The development of identification tools capable of rapidly identifying the O3:K6 strain of *V. parahaemolyticus* is a high priority. Therefore, our research aims to enhance the safety of seafood consumed in the US by developing a rapid strategy for detecting pathogenic *V. parahaemolyticus* in foods, specifically focusing on serotype O3:K6 strains. The ability to distinguish between potentially dangerous isolates and the more prevalent nonvirulent *vibrios* will provide critically important tools that will allow public health workers to develop scientifically based guidelines for identifying water sources more likely to cause infection.

9902650 Pheromone Perception: Structure of Pheromone Binding Protein

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New Investigator Award; Grant 99-35302-8106; $200,000; 3 Years

An understanding of the molecular details of pheromone perception is essential if disruption of mating via sensory inhibition is to be achieved. Chemical interference with the molecular events which occur during pheromone perception in male antenna offers a unique strategy for the disruption of the pre-copulatory behavior of pest insects, and constitutes an environmentally benign alternative to increased use of traditional pesticides. To understand how to produce such "odor blindness", the three dimensional (3D) structures and functions of the proteins involved in the delivery and presentation of these volatile signals to dendritic receptors must be known. Specifically, to unravel the molecular details by which pheromone binding proteins (PBPs) recognize specific pheromone components and display them in such a way as to activate the receptor mediated G protein coupled phospholipase C which leads to ion channel opening by second messenger inositol triphosphate. Complete understanding of protein function and mechanism of action can only be accomplished with a knowledge of its three dimensional structure at atomic resolution.

The specific approach of this proposal is to use high resolution multi-dimensional NMR methods together with distance geometry techniques to determine the 3D solution structure of the pheromone binding protein. This will be accomplished using the best -studied PBP system as a model i.e., the *Anthereae polyphemus* PBP (Apo-PBP).

9902212 Paternal Contributions to the Functional Zygotic Structure in Bovine

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New Investigator Award; Grant 99-35203-7785; $215,000; 3 Years

Mitochondria, the cell's energy-producing factories, carry a small number of genes, which similar to the genes on the cell's chromosomes, encode vital genetic information. The sperm mitochondria and their genes suffer substantial damage during their passage through the female genital tract and are therefore destroyed inside the egg after fertilization. The egg's own mitochondria, in contrast, are kept intact. Although the destruction of sperm mitochondria after fertilization is believed to prevent the perpetuation of damaged, potentially deadly paternal mitochondrial genes, the actual mechanism of this event is not known. Moreover, such a mechanism is not in place when assisted reproductive technologies are used to produce transgenic cattle. Preventing the perpetuation of harmful mitochondrial genes from donor cells will be necessary for successfully using cloning technology without creating defective animals such as the cloned calves reported recently. This project is based on the finding that the small protein ubiquitin, used as a common marker for the destruction of damaged proteins inside cells, specifically marks bull sperm mitochondria for destruction inside the bovine egg. Three specific aims seek answers to the following questions: 1. When are the sperm mitochondria marked by ubiquitin during their development and after fertilization? 2. What is the cellular machinery involved in the destruction of the sperm mitochondria? 3. Which sperm proteins are recognized and marked by ubiquitin? This knowledge has important implications for U.S. agriculture, as it will elucidate the mechanism by which the unwanted mitochondrial genes can be eliminated during natural and assisted fertilization.

9902751 Friction-Damped Energy-Dissipating Timber Connectors: Pilot Tests and Studies

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New Investigator Award; Grant 99-35103-8570; $100,000; 2 Years

Assessment of damage in recent severe earthquakes has demonstrated that there is a clear and pressing need to develop reliable and economical technologies for improving the seismic performance of woodframe and timber structures. The seismic response of timber structures is predominately controlled by their connections, and when they are loaded to failure, damage is concentrated at the joints. Response of timber structures is also highly dependent on their energy dissipation capacity.

Slotted Bolted Connections (SBCs) are simple friction dampers which provide an effective and economical means for controlled, non-destructive energy dissipation in structures subjected to severe seismic loading. Results from proof-of-concept experiments and extensive research on SBCs in steel systems suggest that the potential performance of SBCs in timber joints is very promising. It is believed that SBC friction dampers will prove to be a highly effective means for damage control and energy dissipation in timber structures. SBCs are simple and inexpensive, and lend themselves to a wide range of applications (including retrofit of existing structures.) Use of SBCs will likely lead to safer and better performing wood buildings in areas of high seismic risk.

Experimental work is proposed to test twenty component SBC friction dampers and a single full-scale timber frame with a damped diagonal brace. Damping characteristics and overall performance of tested systems will be analyzed, and numerical models of SBC systems will be developed.

9903341 Germ Line Modification in Farm Animals through Xenogeneic Spermatogonial Transplantation

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New Investigator Award; Grant 99-35205-8620; $300,000; 3 Years

Genetic modification of farm animals is of rapidly increasing importance in agriculture and medicine. The long-term goal of the proposed project is to develop a novel approach to generate transgenic farm animals through modification of the male germ line. In this project, we will recover male germ line stem cells from boars and bulls for transplantation into the testis of recipient mice where they are expected to initiate spermatogenesis of large animal donor germ cells. Experiments are designed to enhance donor cell proliferation and differentiation, and to enrich donor cell populations in stem cells before transplantation. Different culture conditions will be explored to optimize maintenance and proliferation of bovine and porcine germ line stem cells in culture as a basis for their genetic modification. Successful donor spermatogenesis after transplantation of frozen-thawed, cultured, or genetically altered germ cells will allow us to better understand the processes of spermatogenesis in farm animals and subsequently to improve fertility, preserve the genetic heritage of valuable donor animals, and make desirable genetic modifications in the male germ line. Transgenic farm animals expressing desirable genes will show improved production efficiency and disease resistance, reducing the need to use antibiotics and growth promotants which in turn will reduce the risk for residues in the human food supply. In addition, generation of genetically modified pigs as a much needed source of tissues and organs for transplantation to humans with advanced organ failure, and production of pharmaceutical substances in milk can provide potential means of alternate farm income.

9901343 A Grass Endophyte Locus Determining Expression of a Protective Alkaloid

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New Investigator Award; Grant 99-35301-8078; $120,000; 2 Years

Many forage, turf, woodland and meadow grasses host mutualistic fungal endophytes *(Epichloe* and *Neotyphodium* species) that are maternally transmitted (seed borne) and confer to the grasses profoundly enhanced resistance to biotic and abiotic stresses. Among the bioprotective alkaloids produced by endophytes are the lolines (saturated l-aminopyrrolizidines). These unique alkaloids are potent and broad-spectrum insecticides, and sometimes exceed 10,000 g/g plant dry weight. In addition to protection from insects, lolines may have other roles such as enhancing host drought tolerance. Genetic and molecular analyses can provide powerful tests of such roles. I have completed extensive genetic analysis of *Epichloe festucae,* and the results indicate a single genetic locus *(LOL)* that governs expression of lolines. Since I have also identified genetic markers associated with this locus, the next logical step is to construct a contiguous genetic and physical map spanning *LOL.* This map will facilitate development of biological materials necessary to directly test the roles of lolines in agricultural and ecological settings.

9902194 Impacts of a Transgenic Insecticidal Crop on Parasitoid Fitness and Biological Control Performance
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New Investigator Award; Grant 99-35316-7913; $236,370; 2 Years

Transgenic insecticidal crop cultivars (TICs) are cultivars that are genetically engineered to express insecticidal activity against pests. They are predicted to become a major insect management tactic in the next century. Current research on TICs focuses largely on the evolution of pest populations that are resistant to these cultivars, while the potential environmental impacts of TICs have not been examined, including their impact on naturally-occurring biological pest control within and outside transgenic crops. Naturally-occurring biological control relies on a pest's existing natural enemies within a transgenic crop, and is the most important component of integrated pest management (IPM) systems. Moreover, naturally-occurring, biological control relies on the movement of natural enemies between crops; natural enemies destroyed within a transgenic crop cannot provide biological control in other crops. Our research will be the first to examine the potential impacts of a TIC on the performance of parasitoids as natural enemies, and on naturally occurring biological control within and without a transgenic crop: Parasitoids often are the most important natural enemies of pests. The model for this research includes: transgenic and non-transgenic sugarcane, non-transgenic corn, Mexican rice borer (the TIC's target pest), sugarcane borer (a non-target pest under biological control), and *Allorhogas pyralophagus* and *Cotesia favipes* (parasitoids of these pests in sugarcane and corn crops). Our research will, *i*) contribute to the long-term improvement and competitiveness of U. S. agriculture by improving the sustainability of TICs and by understanding their impacts on naturally-occurring biological control, *ii*) advance our understanding of biological control and, *iii*) foster the use of these technologies as components of IPM strategies that do not rely on chemical insecticides.

9902764 Mechanism of Pathogen Survival During Microwave Thermalization
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New Investigator Award; Grant 99-35201-8609; $123,000; 2 Years

Contamination of foods with microorganisms does occur, and cooking by the consumer is one of the last steps in food preparation that will kill microorganisms. Consumers, especially in high-risk groups, increasingly rely on microwave heating to cook or re-heat ready-to-eat foods. It has been reported that microorganisms are more likely to survive in foods cooked using microwaves than foods cooked using conventional methods. This increases the concern for the food safety of microwave cooked foods. Therefore, heating procedures using microwaves that assure product safety should be identified. Recent foodborne disease outbreaks with fresh produce have highlighted the fact that very little research has been conducted on the effectiveness of microwave heating on vegetable products or products that have fresh vegetables as a component. This project will determine the mechanism of microorganism survival during microwave heating; determine the role of fat in the food product on inactivation of the microorganisms; study the effect of the presence or absence of steam on inactivation of the microorganisms; and develop mathematical models to describe the cooking process in the microwave oven in the presence or absence of steam. Specific products to be tested include ground beef at different fat levels; chicken breasts with and without skin; broccoli; and a ready-to eat meal-containing broccoli. This research will result in procedures that can be used to develop microwave-heating procedures that will increase food safety.

9901809 The Role of *SLEEPY1 (SLY1)* in GA Signal Transduction
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New Investigator Award; Grant 99-35304-8103; $120,000; 2 Years

The plant hormone GA stimulates critical processes in plant growth and development including: (i) stem elongation, (ii) flower initiation and fertility, and (iii) germination. This project will clone a gene needed for plants to respond to GA, *SLEEPY1* (*SLY1)*. While much is known about GA hormone biosynthesis, little is known about the mechanisms of GA response, and the GA receptor has not been cloned. Historically, genes encoding hormone receptors have been identified genetically as hormone-insensitive mutants. Attempts to isolate GA-insensitive mutants have been confounded by the fact that they should be unable to germinate. Our novel strategies for the isolation of such GA-insensitive plants recovered ten recessive mutations in *sly1* in the model plant *Arabidopsis*. These are the first recessive GA-insensitive mutants to display the full spectrum of phenotypes seen in severe GA biosynthetic mutants including dwarfism, poor fertility, and failure to germinate. Based on these phenotypes, we hypothesize that *SLY*1 acts early to positively regulate GA response. In order to test this hypothesis we propose to (1) complete the cloning of *SLY1* based on map location, (2) determine its nucleotide sequence, (3) genetically determine the position of *SLY1* in the GA signal transduction pathway, and (4) analyze the *SLY1* gene expression pattern. The *SLY1* gene will provide an important tool for manipulating GA-regulated processes central to the agricultural issues of pre-harvest germination, stand establishment, lodging, and yield.

9901633 The Possibility of Private Crop Insurance and Reinsurance Markets

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New Investigator Award; Grant 99-35400-7755; $34,000; 1 Year

Because of the risk in agricultural production has prevented farmers from engaging in practices that are either more productive, profitable, or sustainable, effective risk management instruments, such as crop insurance, can help to improve productivity, profitability, and sustainability. The Federal government has been facilitating and subsidizing crop insurance to farmers for decades, which has cost tax payers millions of dollars and also provided disincentives to private insurers. An ideal crop insurance that can free the government resources would be one provided by private markets. This proposed research project is devoted to the investigation of the possibilities of such insurance/reinsurance markets both theoretically and empirically. It is believed that high correlations among farm yields have prevented private insurers effectively pooling the risk and providing affordable contracts. This belief is based on the lack of yield independency. A condition which allows for dependency, asymptotic nonpositive correlated distribution, has been found to facilitate risk pooling. The objectives of this project include applying the statistical theory in the content of crop insurance, testing the a.n.c. hypothesis on yields of major crops in the U.S. and /or other countries to support the idea of private insurance and reinsurance markets, and estimating the necessary scale of the insurance pool required by private insurers.

9900736 Predicting Bioavailable Phosphorus Release from Animal Manures
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University of Wisconsin; Department of Soil Science; Madison, WI 53706-1299
New Investigator Award; Grant 99-35107-8263; $96,000; 2 Years

Animal manures are applied to cropland based on rough estimates of nitrogen (N) availability over the growing season. Nitrogen-based application of manure often has led to soil phosphorus (P) overloading where P is susceptible to environmental loss. Concerns related to non-point nutrient pollution from croplands have generated state and federal initiatives to moderate P inputs to soils, including those from animal manures. Our ability to predict bioavailable P release from animal manures is currently hampered by a poor understanding of the factors controlling P mineralization and solubilization. The overall objective of this research is to develop a mechanistic understanding of phosphorus bioavailability from animal manures applied to soils. Specifically we propose to: (1) investigate the biological, chemical and physical factors controlling P release from dairy and poultry manure applied to a variety of soil types at different soil test P levels; (2) identify the P pools in manure that are most important to P mineralization and solubilization using physical fractionation, wet chemistry and spectroscopy (x-ray absorption, XAS, and near infrared) techniques and (3) evaluate the use of near infrared (NIR) spectroscopy as a tool for predicting P release from animal manures. This research should provide a mechanistic understanding of P availability from manures and improve our predictive capabilities.

CONFERENCE GRANTS

The NRI provides partial support to conferences which bring together scientists to identify research needs, update information, or advance an area of research important to U.S. agriculture. Conference proposals are reviewed in the program area most appropriate for the type of research proposed; therefore, these nontechnical summaries also appear under the respective program areas.

9900772 Fourth International Conference on Dietary Assessment Methods

Taren, D.L.
University of Arizona; Prevention Center, Nutrition and Physical Activity Unit; Tucson, AZ 85719
Grant 99-35200-7585; $5,000; 1 Year

The current conference proposal is to support the initial costs of announcements and mailings for the Fourth International Conference on Dietary Assessment Methods. The Conference will be held in Tucson, Arizona from September 17 to September 20, 2000. There is a local organizing committee and an international advisory committee working to 1) develop sessions, 2) identify speakers, 3) participate in sessions, 4) review abstracts, 5) assist with the conference promotion, and 6) obtain financial support. We anticipate an attendance of 500 scientists from about 30 countries. The conference will address several of the “Healthy People 2000" priority areas such as 1) nutrition, 2) maternal and infant health, 3) heart disease and stroke, 4) cancer, and 5) diabetes and chronic disabling conditions. Dietary methods are the cornerstone for conducting epidemiological and intervention studies on diet and health. This conference will have plenary and breakout sessions dedicated to methods for determining the extent of food insecurity and hunger in developed and developing countries, and sessions on measuring the dietary intake of nutrients and non-nutrients that are associated with health. There will be presentations on improved data collection methods and statistical methods for improving analysis of data. Specific conference aims are the following: 1) Provide a forum for sharing new knowledge and ideas; 2) Promote a better understanding of current dietary assessment methodologies; 3) Stimulate interdisciplinary approaches to research; 4) Focus attention on methodological issues; 5) Address methodologic issues of international studies; 6) Identify progress and future research priorities; and 7) Publish the proceedings.

9901785 CO_2-Fixation and Metabolism in Green Plants: Support for Gordon Research Conference

Bohnert, H.J.; Chollet, R.; Foyer, C.
University of Arizona; Department of Biochemistry; Tucson, AZ 85721-0088
Grant 99-35306-7616; $10,000; 1 Year

This proposal asks for support of the September 1999 Gordon Research Conference on "CO_2-Fixation and Metabolism in Green Plants". The conference will be held in Oxford, UK, close to where one of the giants in our field, the recently tragically deceased Tom apRees, began to analyze plant metabolism, inspiring a generation of colleagues. Oxford is a place to pass on the baton to the next generation. We ask for the support of young US scientists to enable them to meet their peers in Europe, because the research field on carbon fixation and metabolism is much more active in Europe than in the US. The reason for this discrepancy is that the field is well-funded in Europe and has attracted much attention from industries that wish to modify starch and oil content of storage tissues.

9903272 FASEB Summer Research Conference on “The Calpain System in Health and Disease”

Goll, D.E.
Federation of American Societies for Experimental Biology; Bethesda, MD 20814
Grant 99-35206-7638; $15,000; 1 Year

This application is for support for a FASEB Summer Research Conference on “The Calpain System in Health and Disease”. The objectives of the conference are: 1)To foster the development of intellectual and collaborative interactions among those individuals working on the calpains, who have come from a wide variety of backgrounds. 2)The conference will provide a forum for the discussion and development of new ideas and approaches to resolving some of the long-standing controversies in the calpain field. The calpain system is critically important to skeletal muscle growth and development, although the exact roles that the calpain system has in skeletal muscle are still unclear. It has been shown that calpain activity is essential for fusion of embryonic skeletal muscle cells. Embryonic muscle lacks calpain activity resulting in no fusion of muscle cells to form mature muscle fibers and hence, no muscle tissue. Other studies have indicated that calpain activity is also essential for cell division or proliferation (impaired proliferation of embryonic muscle cells would lead to fewer cells to fuse and hence very small and/or very few muscle fibers in mature muscle). In growing animals, a variety of evidence has shown that the calpain system is involved in initiating turnover of the myofibrillar or contractile proteins. In this instance, inhibition of calpain activity leads to an increase in the rate of muscle growth. Clearly, new ideas on how calpain activity is regulated, both in developing muscle and in mature muscle fibers, has important implications to skeletal muscle growth and development. This conference will be limited to 140 individuals to facilitate intense and focused discussion of the problems and uncertainties facing the calpain system. It will be the first major international conference devoted to a discussion of the calpain system, and should promote development of new ideas in the calpain field.

9900574 Delivery of Pathogen Signals to Plants
Keen, N.T.; Leach, J.L.; Mayama, S.; Tsuyumu, S.
University of California, Riverside; Department of Plant Pathology; Riverside, CA 92521-0122
Grant 99-35303-8055; $2,000; 1 Year

Plant-Pathogen interactions has been one of the most productive research areas in all of biology in the last several years. Important new information has been obtained on virulence functions in plant pathogens and how these virulence factors are delivered to plant cells. Functions for avirulence gene products are being deduced and many plant disease resistance genes have been cloned and characterized. Of particular interest, the leucine rich repeat domains of these proteins have been shown to convey the specificity for recognition of pathogen elicitors, although it is not yet clear that they are receptors for the elicitors. Progress is also being made in transforming cloned disease resistance genes into new plant species and evaluating them for disease resistance. Finally, progress is occurring in both biochemical studies and mutant hunts for components of intracellular signaling pathways that are involved in active plant disease defense. All of these topics will be discussed by world leaders in their respective fields at the 8th Japan-US Seminar.

9902484 Seventh International Symposium on the Microbiology of Aerial Plant Surfaces
Lindow, S.E.
University of California, Berkeley; Department of Plant and Microbial Biology; Berkeley, CA 94720-3102
Grant 99-35303-8639; $5,775; 1 Year

Research on the microbiology of the phyllosphere has many significant implications for successful crop production and human health worldwide since plants support diverse populations of plant and human pathogenic microorganisms. An understanding of the interactions that occur among phyllosphere microbes and between these microbes and the host plants that they colonize will enable better disease control. Basic aspects of microbial ecology such as gene exchange, habitat modification, and interactions of microbes with the hosts on which they live are also often best examined in this system. To obtain this understanding, communication among scientists from diverse disciplines must be encouraged and fostered. Funds will support the Seventh International Symposium on the Microbiology of Aerial Plant Surfaces, to be held in Berkeley, California from August 3-8, 2000. Objectives of the symposium are: 1. Provide a forum for review and critique of the major findings related to the microbiology of the phyllosphere from 1995 to 2000. 2. Provide a forum in which research in the microbiology of the phyllosphere can be discussed in relation to developments in other areas of microbial ecology, and an arena in which new and current ideas and concepts can be discussed and criticized in this context. 3. Stimulate discussion and speculation as to where future phyllosphere research efforts may encounter the greatest potential gain. 4. Produce a published volume that summarizes progress in the field over the past five years, describes current thinking in the area, and leads toward future progress. 5. Stimulate research in all aspects of the microbiology of the phyllosphere.

9902268 Gordon Research Conference on Fertilization and Activation of Development
Myles, D.G.
University of Rhode Island; Gordon Research Conferences; West Kingston, RI 02892-0984
Grant 99-35203-7608; $2,000; 1 Year

The Gordon Conference on Fertilization and the Activation of Development will be held at the Holderness School, Plymouth, New Hampshire, July 25-July 30, 1999. This conference is concerned with the interaction of the male and the female gametes in the vital process of fertilization and activation of the fertilized egg. There will be nine sessions in the conference. The conference provides a venue for the interaction of biochemists, cell biologists, molecular biologists, physiologists, geneticists and biophysicists investigating fertilization and the activation of development. This conference has been singularly successful in fostering collaborations between investigators with widely disparate experience and seniority. In these ways it is of benefit to the advancement of our understanding of fertilization and the activation of the egg that initiates development of the new organism. Understanding of these phenomena is the basis for improvements in animal breeding (including *in vitro* fertilization, intracytoplasmic sperm injection and cloning).

9902277 20th Annual Meeting of the American Society for Reproductive Immunology
Hansen, P.J.
University of Florida; Department of Dairy & Poultry Sciences; Gainesville, FL 32611-0920
Grant 99-35203-7689; $10,029; 1 Year

Study of interactions between the immune and reproductive systems can have practical implications for animal production by reducing the incidence of bacterial and viral infections of the reproductive tract, increasing fertility and developing methods for regulation of placental growth and hormone secretion. For this project, the USDA NRICGP is supporting the effort by the American Society for Reproductive Immunology (ASRI) to foster the continued development of large animal reproductive immunology by providing speaker travel for the ASRI meeting to be held in June, 2000. In particular, funds are requested to support five of the speakers whose research involves large animal models. The meeting itself will be organized around five plenary sessions. Plenary Session I, entitled "New Concepts in Reproductive

Immunology", will be formatted to highlight some of the recent discoveries that have changed our understanding of mechanisms allowing the fetal allograft to elude the maternal immune system. Plenary Session II will focus on cellular immune function in the reproductive tract, Plenary Session III will be devoted to reproductive microbiology and virology and Plenary Session IV will focus on the regulatory molecules involved in the dialogue between the immune and reproductive systems. The last session (Plenary Session V) is entitled "Nutritional Immunology and Reproduction" and will consist of a series of cutting-edge presentations on an emerging idea that nutritional regulation of immune function can have an impact on reproductive function.

9902394 Evaluating Indirect Ecological Effects of Biological Control: A Symposium Workshop

Hoelmer, K.A., Quimby, P.C.; Kirk, A.A.; Villet, R.
USDA Agricultural Research Service; Beltsville Agricultural Research Center; Beltsville, MD 20705
Grant 99-35316-7912; $5,000; 1 Year.

Biological control is regarded as one of the most environmentally sound methods of pest management, and is increasingly the option chosen whenever possible. However, biological control has been subjected to increasing scrutiny from public interest groups, government agencies and the scientific community, as attention has been focused on the consequences of introduced exotic organisms. Federal policy requires that the impact of biological control programs on non-target organisms, as well non-target pests, be evaluated and optimized. There has been contentious discussion in recent years regarding indirect effects of biological control. This conference will be unique in gathering leading international ecologists and biological control scientists to develop guidelines for improving the science of biological control. Because substantial modern research in ecology, evolutionary biology and theory and practice of biological control has occurred outside of the United States, the international conference in Montpellier, France, will significantly influence future biological control programs in the U. S.

The conference will be held at Agropolis in Montpellier, France, the site of the USDA-ARS European Biological Control Laboratory. Overviews presented by ecologists and biological control scientists will critically assess the status of evaluation efforts. The workshop will then identify and recommend specific ecological approaches for improving biocontrol projects. Sponsors include the International Organization of Biological Control, AGROPOLIS (an international agricultural research and education complex) and the International Centre for Biological Control AGROPOLIS (including the USDA-ARS European Biological Control Laboratory). Subventions have been obtained from U.S. and French organizations.

9900592 Symposium on Metabolic Networking in Plants

Wurtele, E.S.
Iowa State University; Department of Botany; Ames, IA 50011-1020
Grant 99-35100-7586; $5,000; 3 Years

The symposium “Metabolic Networking in Plants” to be held April 22-25, 1999 at Iowa State University, will focus on the interconnections between the pathways that plants use to create and reuse the myriad of chemicals that they contain. Sub-themes are the use of functional genomics in metabolic analyses; impact of environment and development on interconnections between metabolic pathways; the regulation of metabolic pathways; and novel approaches to computer modeling how plant metabolic pathways are integrated. The atmosphere will be informal, and the format allows for extensive discussion both during and between sessions. An important purpose of these symposia is to promote interactions and new collaborations between individuals who might not normally come together at a single symposium. In doing this, we will include a spectra of attendees ranging from world famous scientists to highly promising students and post-doctoral fellows.

As we approach the time that the complete DNA sequence of a plant has been determined, we need to use this wealth of information for defining the intricate metabolic networks. Even if we know the DNA sequence of a gene, we many times do not know its function. We thus need to undertake the task of assigning metabolic functions to batteries of genes on a large scale, identify interrelations among different metabolic pathways and deciphering the interplay in their modulation by environmental and developmental cues. We see the symposium as a way to educate students and post-doctoral associates and to promote meaningful collaborations and interdisciplinary interactions among established scientists.

9902314 Virulence Mechanisms of Bacterial Pathogens International Symposium

Roth, J.A.; Brogden, K.A.
Iowa State University; Department of Veterinary Microbiology and Preventive Medicine; Ames, IA 50011
Grant 99-35204-7720; $9,000; 1 Year

An international symposium entitled Virulence Mechanisms of Bacterial Pathogens will be held September 12-15, 1999 in Ames, Iowa. This meeting will provide an overview of current knowledge of the wide variety of mechanisms used by bacterial pathogens in establishing infection, producing disease, and persisting in the host. Strategies for overcoming bacterial virulence mechanisms will also be presented. The emphasis will be on understanding the mechanisms of host pathogen interactions rather than on current research results. A monograph based on this meeting will be published by the

American Society for Microbiology. The monograph will be entitled Virulence Mechanisms of Bacterial Pathogens, Third Edition, 2000. All meeting registrants will receive a copy of the monograph.

The symposium will be organized under the following topics: Bacterial invasion, colonization, and survival; Bacterial evasion of host defense mechanisms; Bacterial effects on host cell function; and Identification, regulation and transfer of virulence genes. Each major topic will be divided into overview presentations by internationally recognized authorities in the field. Attendees are invited to submit abstracts for poster presentations. The symposium will provide information for molecular biologists, infectious disease researchers, industry scientists, faculty and graduate students interested in bacterial pathogenesis.

9902346 Gordon Conference, Biology of Spirochetes

Zuerner, R.L.; Storm, C.

USDA Agricultural Research Service; National Animal Disease Center; Ames, IA 50010

Grant 99-35204-7787; $9,000; 1 Year

The fourth Gordon Research Conference on the Biology of Spirochetes will be held in January 2000 in Ventura, California. This conference is the only ongoing international meeting devoted to basic research of all medically important and biologically relevant spirochetes. This group of bacteria cause a number of diseases, notably digital dermatitis of cattle, leptospirosis, dysentery in humans and swine, syphilis, Lyme disease, relapsing fever, periodontal disease, and possibly epizootic bovine abortion. As a group, these organisms have historically been difficult to study because many are notoriously difficult to grow in culture, and systems by which their genetics can be studied are in the very early stages of development or are nonexistent. This conference will bring together representatives from laboratories studying all aspects of spirochete biology in an informal forum to discuss cutting-edge research developments. Topics to be included for discussion include physiology, structure, pathogenesis, and immunobiology of these bacteria. The goals of the conference are to foster an environment where current research collaborations are strengthened and new collaborations are developed, and to help define new areas of focus for research in this field. Special attention will be payed to the adequate representation of young investigators at the graduate student, postdoctoral, and junior faculty levels, as well as an appropriate balance in terms of gender and ethnicity.

9901452 The International Workshop on the Regulation of Metabolism, Genetics and Development of the Solvent- and Acid-forming Clostridia

Blaschek, H.P.

University of Illinois; Department of Food Science and Human Nutrition; Urbana, IL 61801

Grant 99-35504-7916; $5,000; 1 Year

The objectives of this bi-annual workshop are to bring together researchers from around the world that are currently working with the biotechnologically important solvent and acid producing clostridia. This meeting is regarded as a truly international meeting whose venue allows for the active participation of a multi-disciplinary group of researchers, from microbiologists and biochemists to engineers interested in molecular genetics and industrial scale-up of the solventogenic clostridia for industrial fermentation. The meeting format will provide an informal setting for comprehensive 30 minute oral research presentations as well as poster sessions. The focus for this upcoming meeting will be to encourage attendance/participation by young scientists, graduate students and post-doctoral fellows. The *Clostridium* VI workshop will take place Friday and Saturday, May 26 and 27, 2000 on the campus of the University of Illinois in Champaign Urbana. This meeting is scheduled to coincide with the ASM General Meeting which will be held in Los Angeles on May 21-25, 2000.

9903365 FASEB Summer Conference: Ubiquitin and Intracellular Protein Degradation

Pickart, C.M.; Vierstra, R.D.

Federation of American Societies for Experimental Biology; Bethesda, MD 20814

Grant 99-35206-7671; $4,760; 1 Year

The conference “Ubiquitin and Intracellular Protein Degradation” will be held from July 31 through August 5, 1999 in Saxton’s River, VT. This meeting is the only regularly-held international meeting devoted to the biology and biochemistry of ubiquitin, a conserved protein ubiquitin which is essential for life in eukaryotes. The meeting is sponsored and partially funded by the Federation of the American Societies for Experimental Biology. The functions of ubiquitin are mediated by its attachment to other proteins in the cell. Its best-understood function is that of a signal which targets proteins for degradation by a novel protease. By setting the levels of key regulatory proteins, the ubiquitin pathway controls fundamental intracellular processes such as the progression of the cell cycle. The pathway also contributes to muscle wasting, a role that is likely to be relevant in large domestic animals. Besides ubiquitin, there exists a distinct family of ubiquitin-like proteins. These proteins seem to function as signals which target their substrates to specific locations within the cell. One notable member of this family, Nedd8, regulates cell cycle progression in mammals and auxin signaling in plants. A better understanding of the role of Nedd8 in the auxin response could allow for improved control of the growth of agriculturally important species. These and

other topics will be addressed in the nine scientific sessions of the 1999 Conference. The Conference should substantially advance our understanding of the uniquitin system and its functions.

9901086 Alliance for Environmental Stewardship: A Comprehensive Approach

Zimmermann, N.G.
University of Maryland, College Park; Department of Animal & Avian Sciences; College Park, MD 20742
Grant 99-35102-8236; $8,000; 1 Year

Nitrogen and phosphorus are nutrients essential for plant and animal life that become pollutants when concentrated. Nutrient pollution is responsible for eutrophication of lakes and bays and high nitrate levels in drinking water associated with blue-baby syndrome. Nutrients released into the environment are produced by (1) animal agriculture, (2) commercial fertilizer, (3) biosolid residuals (sludge) from human and animal waste treatment facilities, (4) urban components, and (5) on-site wastewater/sewage treatment (septic tanks). Sixty-one speakers and 125 invited key personnel that equally represent each of these sources, and the public, will have a comprehensive, objective, and science-based discussion of the impact on our environment by nutrients at a meeting titled, "Alliance for Environmental Stewardship: A Comprehensive Approach," on September 27-29, 1999, in St. Louis, MO. Workshop goals include: (1) provide an opportunity to cooperate with representatives for all sources of nutrients, and persons who influence policy (e.g., media) to discuss ways in which we can work together to protect the environment; (2) create recognition that all sources of nutrients impact the environment; (3) promote sound decision making processes that require scientific and comprehensive evaluation; (4) define and implement proactive programs through commercial agencies, government agencies and universities; and (5) develop a set of prioritized recommendations that decision makers may use as a basis for comprehensive policy development, and establishment of research and economic priorities that will correct or ameliorate pollution problems. Papers, poster abstracts, and prioritized recommendations published in the proceedings, will be available to the public.

9903292 Conference for the International Society of Animal Genetics

Schook, L.B.
University of Minnesota, Twin Cities Campus; Department of Veterinary Pathobiology; St. Paul, MN 55108-6010
Grant 99-35205-8557; $10,000; 1 Year

Our primary objective is to organize the 27th International Conference on Animal Genetics, which will contribute to the discovery and dissemination of knowledge of animal genomics to serve food, agricultural, and ecological sectors of global societies. The International Society for Animal Genetics (ISAG) evolved from a series of annual workshops, to a society focusing on basic and applied research within the fields of immunogenetics, biochemical genetics, genomics and molecular genetics. The society's purposes are: (a) to encourage the study of genetically influenced characters of animal tissues and fluids; and (b) to facilitate the exchange of ideas and materials between research workers. In particular, the Society is proud of its international membership, which contributes diverse approaches to promoting animal genetics. Since 1954, ISAG has held its International Conference on Animal Genetics every 2 years at different locations around the world. Recent meetings in Tours, France (1996), and Prague, Czech Republic (1994), attracted over 500 scientists and students. Attendance at the year 2000 meeting is expected to be significantly greater than that of the very rewarding 1998 Auckland, New Zealand meeting (600), because of the meeting's accessible North America location. The aim of the meeting is to foster the interaction and exchange of information between all researchers in the field of genetics of farmed and companion animal species (from fish to horses); this is supportive of the aims of the recently developed U.S.D.A. Agricultural Genome Initiative.

9903502 12th International Congress on Nitrogen Fixation

Vance, C.P.
University of Minnesota; USDA Agricultural Research Service; Midwest Area; Plant Science Research Unit; St. Paul, MN 55108
Grant 99-35305-8405; $8,000; 1 Year

Nitrogen is one of the most important elements needed by crops for maximum yield and quality. Symbiotic nitrogen fixation by legume plants, such as alfalfa and soybean, plays a critical role in sustainable agriculture by providing 90 million metric tons of nitrogen each year. This process occurs in small wart-like structures called nodules on legume roots. Symbiotic bacteria inside the nodule convert atmospheric nitrogen gas into a form that the legume plant can use. Therefore, legumes need little if any nitrogen fertilizer. Moreover, legumes can provide significant nitrogen to other crops like wheat and corn when grown in rotations. By growing legumes farmers can reduce the amount of nitrogen fertilizer they need to purchase and apply. In addition, the nitrogen gained through symbiotic nitrogen fixation is less likely to contaminate ground- and surface water. Every two years an international conference is held which brings together scientists from throughout the world doing research on nitrogen fixation. This conference allows scientists from the United States to discuss and report on current scientific advances made in the science of nitrogen fixation. The 12th International Congress

on Nitrogen Fixation will be held in Brazil. The $8,000 awarded by the USDA/NRI Program will be used to help pay in-country expenses for 12 U.S. scientists attending the Conference.

9901654 Sixth Symposium on Lactic Acid Bacteria: Genetics, Metabolism, and Applications
Klaenhammer, T.R.
North Carolina State University; Department of Food Science; Raleigh, NC 27695-7624
Grant 99-35503-8118; $8,000; 1 Year

Funds are requested in support of the Sixth Symposium on Lactic Acid Bacteria -Genetics Metabolism and Applications to be held in Veldhoven, the Netherlands, on September 19-23, 1999. This triannual international meeting is held on the lactic acid bacteria (LAB), microorganisms which are intimately involved in food fermentation and constitute a major portion of the human diet since ancient times. LAB play an essential role in the preservation of raw food and contribute to the nutritional, organoleptic and health-promoting properties of foods for humans and animals. Beyond their traditional roles, the LAB are now being exploited for a variety of medical, pharmaceutical, and biotechnological applications; for example expression of vaccines, antimicrobials, industrial enzymes, and bioactive compounds. These old and new critical roles for LAB have lead to an explosion of scientific interest and industrial activity over the past 20 years. The full invited lectures and extended abstracts of the other lectures will be published in a special issue of Antonie van Leeuwenhoek. The number of participants in the conference has increased from 175 in 1983 to 540 in 1996, with US representation near 6% of the participants over the 16 year history. U.S. representation in 1999 is among the highest in the history of the conference, with five of seventeen plenary lectures to be given by U.S. speakers. This proposal requests $8,000 in funds from the NRIGCP value-added program to aid in the support of expenses for US speakers who will present plenary lectures at the 6th LAB symposium.

9901808 Research Conference 'Auxin 2000'
Jones, A.M.
The University of North Carolina, Chapel Hill; Department of Biology; Chapel Hill, NC 27599-3280
Grant 99-35304-8001; $6,000; 1 Year

The plant hormone auxin is ubiquitous among plant species and has a key role in most aspects of plant growth and development, including those that are directly related to crop performance. During the last few years, the application of new analytical, biochemical and genetic approaches has led to dramatic advances in our understanding of auxin metabolism, transport and action. At the same time, the availability of the complete genome sequence of *Arabidopsis*, and perhaps other, plant species within the next few years will accelerate the rate of discovery. For these reasons, we are planning an international meeting of biologists working on all aspects of auxin biology. The meeting, the first of its kind and to be named "Auxin 2000", will be held in May 2000 on the island of Corsica. Our objective is to bring the very best researchers in the field together in an atmosphere conducive to formal presentation of the latest research results as well as informal discussion and consultation. To promote interaction, attendance at the meeting will be limited to 120 scientists. Approximately 45 of the attendees will be invited speakers. The remainder will be selected by the organizers from a pool of applicants. Approximately 40 places will be reserved for postdocs and senior graduate students. We request funds for a small fraction of the travel cost of the invited US speakers and for fellowships to US postdocs and senior graduate students to participate.

9900702 Federation of American Societies for Experimental Biology Summer Research Conference on Calcium Oxalate in Biological Systems
Holmes, R.P.
Wake Forest University School of Medicine; Department of Urology; Winston-Salem, NC 27157
Grant 99-35200-7592; $5,000; 1 Year

Funds were requested to provide partial support for a FASEB Summer Research Conference entitled Calcium Oxalate in Biological Systems to be held at Copper Mountain, CO, on August 15-20, 1999. This conference will bring together basic scientists and clinician scientists from a variety of disciplines whose research deals with calcium or oxalate or some aspect of their interaction to form crystals. Such scientists will include plant biologists who investigate the orderly formation of calcium oxalate crystals of precise shape in specialized plant cells, microbiologists who examine the ability of microorganisms to metabolize oxalate, nutritionists who study the absorption and bioavailability of dietary calcium and oxalate, physiologists who examine the transport of calcium and oxalate and their interaction with organs, biochemists and cell biologists who investigate aspects of their intracellular function and metabolism, and clinical scientists who examine the pathophysiology of calcium oxalate crystallization, particularly in the formation of kidney stones. The goals of the Conference are: 1) to identify issues of consensus and controversy; 2) to promote a cross-fertilization of ideas; 3) to provide opportunities that facilitate scientific collaborations; and 4) to promote scientific exchange of information, ideas and methodologies. Nine scientific sessions will be held over a 5 day period. Questions that should arise in the conference

include the functional role of oxalate and calcium oxalate in plants, environmental factors that influence calcium and oxalate levels in plants, the bioavailability of these plant components for absorption, and the nature of the absorption processes.

9901743 Gordon Research Conference on the Biochemical Aspects of Photosynthesis
Golbeck, J.H.
The Pennsylvania State University; Department of Biochemistry and Molecular Biology; University Park, PA 16802
Grant 99-35306-7543; $12,000; 1 Year

This proposal requests support for a Gordon Research Conference on "Biochemical Aspects of Photosynthesis" to be held at the New England College, New Hampshire from June 13 to 18, 1999. The conference Chair is Dr. John H. Golbeck and the Vice-Chair is Dr. Gary Brudvig. Advances in the interdisciplinary field of photosynthesis are reflected in the core talks at the 1999 Conference in sessions devoted to the biochemistry and biophysics of Type I (Photosystem I, green sulfur bacterial and heliobacterial) reaction centers, Type II (Photosystem II, purple bacterial) reaction centers, cytochrome complexes, and light-harvesting antenna. An emerging research area which is yielding impressive results is the biogenesis, assembly, and turnover of the photosynthetic apparatus. Two sessions are devoted to this topic, including one on the environment and assembly of the manganese cluster in photosystem II and the other to the development and turnover of Type I and Type II reaction center complexes. An entire session is devoted to new information gleaned from the now-complete gene sequences of the anaerobic green sulfur bacterium *Chorobium tepidum* and the purple non-sulfur bacterium *Rhodobacter capsulatus*. Lessons learned from the natural photosynthetic complexes along with advances in organic photochemistry have led to the synthesis of artificial photosystems, and the conference includes a session devoted to biomimetic systems and protein engineering. The 1999 conference ends with an outside perspective on lessons learned from the assembly of the iron-molybdenum cofactor in the nitrogenase enzyme.

9902141 Veterinary Virology Symposium: Comparative Mechanisms of Viral Pathogenicity and Immunity
Benfield, D.A.
South Dakota State University; Department of Veterinary Science; Brookings, SD 57007-1396
Grant 99-35204-7862; $5,500; 1 Year

This proposal requests funds for the First Veterinary Virology Symposium on “Comparative Mechanisms of viral Pathogenicity and Immunity”. Viruses are a major cause of morbidity and mortality in food and companion animals. The major economic impact of virus infections include poor growth, loss of reproductive capacity, cost of treating secondary bacterial infections, death of the animal and emotional suffering of producers (result of income loss) and pet owners (loss of companion). In addition, new viruses and viral diseases continue to emerge. Examples of emerging viral diseases include porcine reproductive and respiratory syndrome, bovine virus diarrhea virus type 2, bovine respiratory coronavirus, porcine respiratory coronavirus, avian H1N1 influenza viruses in pigs, canine parvovirus type 2 and circovirus, the putative agent of porcine postweaning multisystemic wasting syndrome. One of the most effective means to bring experts in different disciplines together is through a conference or symposium format. This format allows scientists, veterinary virologists, to acquire new knowledge from colleagues, exchange ideas, compare research data and foster new collaborations in research. Comparative studies of viruses will in the long-term result in novel approaches for the control and elimination of viral diseases. This collaborative approach to the study of animal viruses may result in: 1) treatments, management protocols or control methods that improve the health and well-being of food and companion animals; 2) an improved income for food animal producers; 3) a safer food supply; and 4) the emotional well-being of companion animal owners.

9900643 Tenth International Symposium on Iron Nutrition and Interactions in Plants
Grusak, M.A.
USDA Agricultural Research Service; Children’s Nutrition Research Center; Houston, TX 77030
Grant 99-35100-7591; $8,389; 1 Year

This grant provides partial support for the 10th International Symposium on Iron Nutrition and Interactions in Plants, to be held in Houston, Texas, May 14 - 19, 2000. The objective of this meeting is to bring together scientists from a broad range of disciplines, in order to provide a forum for researchers to exchange new knowledge, ideas and techniques, and to provide stimulus for future collaborations. Plant iron nutrition is an important field that has relevance not only to crop health and productivity, but also to the nutritional content of harvested foods for human and animal consumption. Plant iron deficiency, in particular, is a national and international concern, because of the numerous soil types and soil conditions which limit iron availability. Because a plant’s ability to respond to this environmental stress can involve a number of physical and biological processes, this continuing Symposium in previous years has attracted an international audience of soil scientists, microbiologists, agronomists, plant physiologists, biochemists, molecular biologists, breeders, ecologists, and human nutritionists. In keeping with this range of interests, the scientific program for the 10th International Symposium will span the soil -- microbial -- plant -- human continuum, in order to foster connections and understanding between processes occurring at various physical and biological levels. Both oral and poster presentations will be integrated to educate

and challenge the attendees. The outcome of this Symposium will be to enhance our collective knowledge of plant iron nutrition, and the ways in which plants can respond to iron-deficiency stress.

9902716 Gordon Conference on Metamorphosis
Riddiford, L.M.
Gordon Research Center; University of Rhode Island; West Kingston, RI 02892-0934
Grant 99-35302-8250; $5,448; 1 Year

This grant provides partial support for the first Gordon Conference on Metamorphosis July 10-15, 1999 at Connecticut College, New London, CT. The Conference brings together people working on metamorphosis in insects and amphibians and a variety of other organisms to discuss the common cellular and molecular events that occur in metamorphosis and the questions that it poses about coordination and control systems in development. The 8 scientific sessions deal with hormonal regulation of metamorphosis at both cellular and organismal levels, mechanisms of gene activation and repression, intracellular receptors and gene regulation, transcription factor cascades in metamorphosis, the evolution of metamorphosis, cellular specification and differentiation during insect metamorphosis, cellular alterations during amphibian metamorphosis, and cell death during metamorphosis. There are 27 invited speakers and 21 contributed posters with 6 of the latter presenters (4 of whom are graduate students or postdoctoral researchers) invited to give short talks on their provocative results. The cross-fertilization among the workers on the various metamorphic systems should foster new insights into these complex systems and the similarities and differences between vertebrate and insects at the molecular level. These insights can lead to an elucidation of the critical steps that may be utilized as possible intervention points for control of insect and nematode pests of U. S. agriculture.

9902384 Annual Meeting of the Society for the Study of Reproduction
Griswold, M.D.
Society for the Study of Reproduction; Madison, WI 53711-2021
Grant 99-35203-7805; $16,000; 1 Year

The 1999 annual meeting of the Society for the Study of Reproduction is organized around the theme of, “Research in Reproduction in the New Millennium”. In the new millennium, issues involving animal and human reproduction will assume a central worldwide importance. Some of these issues include: greater efficiency of livestock reproduction, decreased fertility in humans and animals, preservation of endangered species and wildlife resources and novel approaches to control reproduction. To address these issues, it is essential to advance our fundamental understanding of the underlying processes at the molecular and biological levels. In the new millennium, the importance of ruminant livestock in providing human food will assume more importance. While the entire scientific sessions will be of interest to scientists working in reproduction in food animals, three minisymposia are designed to focus on reproduction in domestic animals: 1)Sexual Differentiation, 2)Cellular & Molecular Regulation of Luteal Formation, Function and Regression, and 3)Science and Applications of Animal Cloning. Participants in this meeting include academic, Federal and industrial scientists, postdoctoral fellows and graduate students. All speakers in the plenary sessions or the minisymposia are encouraged to submit review articles for publication in *Biology of Reproduction*, the leading journal in the reproductive sciences.

U.S. RICE GENOME SEQUENCING PROJECT (USDA, NSF, DOE)

Division Director - Dr. Ed Kaleikau, USDA

This competitive grant program is part of the National Plant Genome Initiative (NPGI) coordinated by the National Science and Technology Council (NSTC)--the long-term goal of this interagency effort is to understand the structure and function of genes in plants important to agriculture, environmental management, energy and health. As a collaborative, interagency effort, the Cooperative State Research, Education, and Extension Service (CSREES) of the Department of Agriculture, the National Science Foundation, and the Department of Energy solicited proposals to initiate systematic sequencing of the genome of rice in the United States as part of an international effort that includes the Rice Genome Program of Japan. The ultimate goal of this project is to sequence the entire rice genome as a model monocot (grass) species. When completed, scientists will have a resource for understanding the functions and relative map locations of all cereal genes; the use of map-based sequence information to identify and provide markers for agronomically significant genes; the molecular basis of plant growth and development so that fundamental questions in plant physiology, biochemistry, cell biology, and pathology can be addressed and; the relationship of genome structure to gene expression.

9904378 Sequencing of Chromosome 10 of Rice and Validation of Annotation Methods for Rice

Buell, C.R.; Benito, M.; Lin, X.
The Institute for Genomic Research; Rockville, MD 20850

Grant 99-35317-8275; $7,200,000 ($3,450,000 - USDA; $3,450,000 - NSF; $300,000 - DOE);
3 Years

Rice is considered a model species for monocotyledonous plants such as the cereals maize, wheat, sorghum, and barley. Rice has a relatively small genome, is diploid, is readily transformable and has tractable genetics that include diverse germplasm. These attributes, in addition to its role as a major food source for a majority of the world's population, have resulted in the development of genetic and molecular resources focuses on obtaining the complete genome sequence of rice. Not only will the complete sequence of rice provide a reservoir of genes by which to understand rice growth and development, but it will also provide a set of molecular tools to leverage sequence information to highly related species such as maize, wheat and barley that are more recalcitrant to genomic approaches. A total of 31.5 Mb of rice genomic sequence will be generated and released to Genbank. We will annotate this sequence using gene prediction programs, identification of related genes within the database, and characterization of the repetitive structures/sequences. In addition, links between rice sequences and sequence, physical, and genetic information from other plant species will be generated.

9904375 DNA Sequencing of Rice Chromosomes 3 and 10

Wing, R.A.; McCombie, R.W.; Wilson, R.
Clemson University; Genomics Institute; Clemson, SC 29634-5708

Grant 99-35317-8505; $5,100,000 ($2,550,000 - USDA; $2,550,000 - NSF); 3 Years

The cereals are the most important food crops in the world and determining the entire genomic sequence of a model cereal will reveal the basic gene set from monocot plants. In this proposal we have assembled an experienced and productive team to participate in an international effort to determine the genomic blueprint of rice. The objective of this proposal, described below, is to produce and annotate approximately 20 Mb of contiguous rice genomic sequence from chromosome 10 in three years. The overall project will be managed by Clemson University (CUGI), in collaboration with Cold Spring Harbor Laboratory (CSHL), and the Washington University. Clemson will hire a Project Manager to coordinate the overall project operations.

1) 160 130kb Nipponbare BAC clones anchored chromosome 10 will be validated for insert integrity and chromosome location by CUGI. Shotgun library development and production sequencing of the 160 BAC clones will be performed at CSHL (64%) and CUGI (33%).

2) Finishing and automated annotation will occur at CUGI and CSHL.

3) WUGSC will lead a group in finishing technology development aimed at establishing robust chemistries that can be used by the consortium to finish difficult regions of the rice genome.

4) All sequence date will be immediately released to community databases (Genbank HTGS section) according to the "Bermuda" policies. In addition, we will publish our results in scientific journals and electronically where annotated BAC clones will be displayed through a common web interface maintained at CUGI.

APPENDICES

APPENDIX A.
Statistics for the 1999 Fiscal Year

Note: This is not a budgetary document and contains information only about competing awards. Noncompeting awards (e.g., continuation awards) appear only in the Abstracts of Funded Research for the year initially funded. Full budgetary reporting is available each year in the NRI Annual Report.

TABLE I

Submission and Award Statistics for NRI, Fiscal Year 1999
Unobligated Funds[a]

Type	Number of Submissions	Number of Grants	Total Dollars Awarded
New Proposals	1,872	403	$62,810,588
Resubmitted Proposals	530	134	22,456,927
Renewal Proposals	273[b]	123[c]	24,084,493
Conference Proposals	44	25	189,101
OVERALL[d]	2,719	685	$109,541,109

[a] Funds available for new grants
[b] Includes 16 renewal proposals that were resubmissions
[c] Includes 6 renewal awards that were resubmissions
[d] Excludes 3 interagency awards (made as part of the U.S. Rice Genome Project and as part of the *Arabidopsis thaliana* Genome Sequencing Project).

TABLE II

Fund Allocations for NRI, Fiscal Year 1999
Unobligated Funds[a]

Research Area/Program	Number of Grants	Total Dollars Awarded
NATURAL RESOURCES & ENVIRONMENT		
Plant Responses to the Environment[2]	24	$3,698,389
Ecosystem Science	19	4,871,500
Water Resources Assessment and Protection	24	4,420,500
Soils and Soil Biology	19	4,257,500
NUTRITION, FOOD SAFETY, & HEALTH		
Improving Human Nutrition for Optimal Health[3]	26	4,625,233
Food Safety	25	4,061,942
Epidemiological Approaches to Food Safety	9	5,312,072
ANIMALS		
Animal Reproductive Efficiency	29	4,554,818
Animal Health and Well-Being[4]	57	11,140,731
Animal Genome and Genetic Mechanisms	20	6,076,755
Animal Growth, Development, and Nutrient Utilization	18	3,226,346
PEST BIOLOGY AND MANAGEMENT		
Entomology and Nematology	43	6,395,000
Plant Pathology	32	5,017,815
Biologically Based Pest Management	15	2,310,675
Weed Biology and Management	9	1,312,188
PLANTS		
Plant Genome	19	4,324,913
Plant Genetic Mechanisms	28	4,518,500
Plant Growth and Development	35	5,508,000
Nitrogen Fixation/Nitrogen Metabolism	15	2,300,000
Photosynthesis and Respiration[5]	16	2,271,020
MARKETS, TRADE, & RURAL DEVELOPMENT		
Markets and Trade[6]	24	1,993,000
Rural Development	14	1,700,000
ENHANCING VALUE AND USE OF AGRICULTURAL AND FOREST PRODUCTS		

Food Characterization/Process/Product Research[7]	27	3,949,541
Non-Food Characterization/Process/Product Research	17	2,344,605
Improved Utilization of Wood and Wood Fiber	19	2,561,018
OTHER		
Agricultural Systems	16	3,507,366
Strengthening Programs	88	3,426,535
Arabidopsis thaliana Genome Sequencing Project - Interagency[8]	1	100,000
U.S. Rice Genome Project - Interagency	2	2,000,000
Awards to be Determined[9]		880,713
GRAND TOTAL[10]	690	112,666,675

[1]The content of this table varies slightly from tables provided in documents supporting the President's budget to Congress each year in the following ways: 1) while the documents supporting the President's budget includes data only for funds from the 1999 appropriation, this table includes data on all awards from proposals submitted to the 1999 proposal cycle, regardless of the source of funds (as noted in the table) and 2) awards are arranged in this table under program area (to which proposals are submitted and reviewed) as opposed to relationship to appropriated budgetary lines.
[2]Includes 8 awards funded in whole or part with $1,603,584 from the FY 1998 appropriation.
[3]Includes 1 award funded in part with $4,679 from the FY 1998 appropriation.
[4]Includes 1 award funded in part with $22,155 from the FY 1998 appropriation.
[5]Includes 2 awards funded in whole or part with $293,664 from the FY 1998 appropriation.
[6]Includes 1 award funded in part with $3,091 from the FY 1998 appropriation.
[7]Includes 1 award funded in whole with $185,000 from the FY 1998 appropriation.
[8]Awarded through an interagency transfer to the National Science Foundation.
[9]As of November 27, 1999
[10]Includes 13 awards funded in whole or part with $2,109,082 from the FY 1998 appropriation.

TABLE III

Agricultural Research Enhancement Awards, Fiscal Year 1999
Unobligated Funds[a]

Type	Number of Grants	Total Dollars Awarded
Postdoctoral Fellowships	20	$1,744,503
New Investigator Awards	38	5,422,328
Strengthening Awards		
Research Career Enhancement Awards	6	331,524
Equipment Grants	44	1,231,517
Seed Grants	38	1,863,494
Standard Strengthening Research Projects	63	8,340,112

[a] Funds available for new grants

APPENDIX B.
Scientists Who Served on NRI Peer Review Panels for Fiscal Year 1999

Thomas Adams	University of California
Michael Adang	University of Georgia
James R. Alfano	University of Nevada
Ronald Allen	University of Arizona
William Amponsah	North Carolina A&T State University
Haejung An	Oregon State University
Jay Anderson	Idaho State University
Stephen H. Anderson	University of Missouri
Michael Anta	University of Hawaii
Russell V. Anthony	Colorado State University
Gerald T. Babcock	Michigan State University
C. “Rick” Barb	USDA/ARS Russell Research Center
Mary E. Barbercheck	North Carolina State University
Ann H. Barrett	CFP Natick Soldier Center
Andreas Baumler	Texas A&M University
Fuller W. Bazer	Texas A&M University
Steven Buccola	Oregon State University
Randolph Beaudry	Michigan State University
Patricia A. Bedinger	Colorado State University
James K. Belknap	Auburn University
Leonard N. Bell	Auburn University
Judith L. Bender	Johns Hopkins University
David A. Benfield	South Dakota State University
Tracy L. Benning	University of California
Richard A. Bessen	Creighton University
Curtis E. Beus	Washington State University
Arvind Bhagwat	University of Maryland
Marla L. Binzel	Texas A&M University
Samuel J. Black	University of Massachusetts
Carol D. Blair	Colorado State University
R. W. Blancher	University of Missouri
Anthony B. Bleecker	University of Wisconsin
Jeff Bloomquist	Virginia Polytechnic Institute
Linda S. Boeckner	University of Nebraska
Terry L. Bowersock	Pharmacia and Upjohn
Joe Bozell	National Renewable Energy Laboratory
Terrence M. Bradley	University of Rhode Island
Clark Brekke	University of Tennessee
Stephen Broome	North Carolina State University
K. Shane Broughton	University of Wyoming
Wendy Catherine Brown	Washington State University
Kaye L. Brubaker	University of Maryland
Judith Ann Brusslan	California State University

Gerald M. Buening	University of Missouri
Douglas D. Buhler	USDA-ARS National Soil Tilth Laboratory
Fitzroy D. Bullock	University of Tennessee
Ben Burr	Brookhaven National Laboratory
Bruce C. Campbell	USDA-ARS
John L. Capinera	University of Florida
John Cardina	The Ohio State University
Vicki Chandler	University of Arizona
Yung-Fu Chang	Cornell University
Ralph E. Charlton	Kansas State University
Weixin Cheng	Louisiana State University
David J. Chitwood	USDA-ARS
Paul Chomet	DEKALB Genetics Corporation
Shafiqul I. Chowdhury	Kansas State University
Lynda M. Ciuffetti	Oregon State University
Steven Clark	University of Michigan
Margot Cleary	University of Minnesota
Albert Clemmens	USDA-ARS
Archie C. Clutter	Oklahoma State University
Joel Coates	Iowa State University
Robert Collier	Monsanto Company
Anthony W. Confer	Oklahoma State University
Andrea May Cooper	Colorado State University
Thomas A. Costello	University of Arkansas
Thomas A. Coudron	USDA-ARS
Maribeth A. Cousin	Purdue University
Wendell Cropper	Rosenstiel School of Marine & Atmospheric Science
James Cuda	University of Florida
Karen W. Cullen	University of Texas
Catherine Nettles-Cutter	USDA-ARS
Richard J. Cyr	Pennsylvania State University
Robert Dailey	West Virginia University
Douglas Darling	University of Louisville
Ashim K. Datta	Cornell University
Jessica G. Davis	Colorado State University
R. Kelly Dawe	University of Georgia
Jeffrey F.D. Dean	University of Georgia
Evelyn Dean-Nystrom	USDA-ARS
Michael J. Delwiche	University of California
Tarlochan Dhadialla	Rohm and Haas Company
Martin B. Dickman	University of Nebraska
Biao Ding	Oklahoma State University
Victor J. DiRita	University of Michigan Medical School
Richard A. Dixon	Samuel Roberts Noble Foundation
John S. Doctor	Duquesne University
Rebecca W. Doerge	Purdue University

Roy H. Doi	University of California
Katherine M. Donato	Louisiana State University
Xinnian Dong	Duke University
C. Richard Dorn	Ohio State University
Robin A. Douthitt	University of Wisconsin
Robert Duby	University of Massachusetts
Patricia A. Duffy	Auburn University
Gerald E. Duhamel	University of Nebraska
William E. Dyer	Montana State University
William B. Eggleston	Virginia Commonwealth University
Sanford D. Eigenbrode	University of Idaho
Cady R. Engler	Texas A&M University
Hollis Erb	Cornell University
Immaculata Estevez	University of Maryland
Robert J. Etches	Origen Therapeutics
Jon M. Faubion	American Association of Cereal Chemists
Christiane Fauron	University of Utah
Robert M. Faust	USDA-ARS
Mark J. Federspiel	Mayo Clinic Foundation
Gary M. Felton	University of Maryland
Scott E. Fendorf	Stanford University
Maria Luz Fernandez	University of Connecticut
Rene Feyereisen	University of Arizona
Patricia Flebbe	USFS Southern Research Station
Norma L. Fowler	University of Texas
Raymond C. Francis	SUNY Environmental Science & Forestry College
Gary R. Fulcher	University of Minnesota
Michael D. Gale	John Innes Centre
Gary M. Gardner	University of Minnesota
H. Rex Gaskins	University of Illinois
Laurel J. Gershwin	University of California
Donald D. Gilbert	Indiana University
Jane Glazebrook	Novartis
W. Richard Goe	Kansas State University
John H. Golbeck	Pennsylvania State University
David A. Golden	University of Tennessee
Peter B. Goldsbrough	Purdue University
Thomas Gomez	USDA-APHIS-VS
Hassan Gourama	Pennsylvania State University
Lourdes Gouveia	University of Nebraska
James K. Graham	Colorado State University
Stewart M. Gray	Cornell University
James I. Grieshop	University of California
Dennis C. Gross	Washington State University
Elizabeth Guertal	Auburn University
Mark Guiltinan	Penn State University
Cameron Hackney	Virginia Polytechnic Institute & State Univ.

Darryl L. Hadsell	Baylor College of Medicine
Stephen W. Hallgren	Oklahoma State University
Brian D. Halligan	Medical College of Wisconsin
L. Curtis Hannah	University of Florida
Kevin M. Hannon	Purdue University
Margaret Hardin	National Pork Producers Council
William L. Hargrove	Kansas State University
Edward D. Harris	Texas A&M University
Linda J. Harris	University of California
Robert D. Harter	University of New Hampshire
Michael Havercamp	University of Nevada
Jane L. Hayes	USDA Forest Service
Scott A. Heckathorn	College of Charleston
Sabine Heinhorst	University of Southern Mississippi
Gregg Henderson	Louisiana State University
James W. Hendrix	University of Kentucky
John Herbold	University of Texas
Hildegarde Heymann	University of Missouri
Arthur Hinton Jr.	USDA-ARS-RBRRC
David W. Hird	University of California
Jason Hlywka	University Nebraska
Margo Holland	Michigan State University
Robert Holland	Michigan State University
David W. Horohov	Louisiana State University
James Lee Howard	USDA Forest Service
Gregg A. Howe	Michigan State University
Casey W. Hoy	OARDC
You-Lo Hsieh	University of California
Samuel J. Huang	University of Connecticut
Samuel Hudson	North Carolina State University
Ray Huffaker	Washington State University
Scott Hulbert	Kansas State University
Sheila I. Hull	Baylor College of Medicine
Laura Hungerford	University of Nebraska
Susan Hutson	Wake Forest University
Keith T. Ingram	University of Georgia
Erin E. Irish	University of Iowa
Louise E. Jackson	University of California
Jay-Lin Jane	Iowa State University
Barry W. Jesse	Rutgers University
Yan Jin	University of Delaware
Lynn Joens	University of Arizona
Rodney W. Johnson	University of Illinois
Sally E. Johnson	Pennsylvania State University
William T. Johnson	USDA-ARS
Howard S. Judelson	University of California
Stephen L. Kaattari	College of William and Mary
Seogchan Kang	The Pennsylvania State University
Jeffrey Karns	USDA-ARS-BA-SMSL

Nancy P. Keller	Texas A&M University
Darshan Kelley	USDA-WHNRC-ARS
Thomas D. Kocher	University of New Hampshire
Alan K. Knapp	Kansas State University
Sung I. Koo	Kansas State University
Roger Alan Korus	University of Idaho
K. Gus Kousoulas	Louisiana State University
Christine A. Kozak	NIH-NIAID-LMM-VBS
Timothy J. Kring	University of Arkansas
Vera A. Krischik	University of Minnesota
Beth Krizek	University of South Carolina
K. J. Samuel Kung	University of Wisconsin
Gael Kurath	Western Fisheries Research Center
Elemer M. Lang	West Virginia University
Nora Lapitan	Colorado State University
Robert P. Larkin	University of Maine
Roland M. Leach	Pennsylvania State University
Robert Leichti	Oregon State University
John A. Leigh	University of Washington
Henry K. Leung	Frito-Lay, Inc.
Jay F. Levine	North Carolina State University
Stephen J. Libby	North Carolina State University
Stephen P. Long	University of Illinois
George Loo	University of North Carolina
Christopher A. Makaroff	Miami University
Stephen P. Mayfield	The Scripps Research Institute
Matthew Lucy	University of Missouri
Mona C. Mehdy	University of Texas
Kojo Mensa-Wilmot	University of Georgia
Bruce A. McPheron	Pennsylvania State University
Michael McClelland	Sidney Kimmel Cancer Center
Karen McDowell	University of Kentucky
John J. McGlone	Texas Tech University
Keith C. McGregor	USDA National Sedimentation Laboratory
Michael McMullen	University of Missouri
Sally A. Miller	Ohio State University
F. Christopher Minion	Iowa State University
Debra Mohnen	University of Georgia
Roberta Morales	University of Maryland
Bruce A. Morgan	Harvard Medical School
Mark Morrison	University of Nebraska
Rodney A. Moxley	University of Nebraska
Peter J. Myler	Seattle Biomedical Research Institute
Gloria K. Muday	Wake Forest University
Richard Mural	Oak Ridge National Laboratory
Ronald T. Nagao	University of Georgia
Jonathan Neal	Purdue University
Ruth Catriona Newberry	Washington State University
John N. Nishio	University of Wyoming

Jan P. Nyrop	Cornell University
Kenneth Odde	Pfizer
Jack Odle	North Carolina State University
David Orden,	Stanford University
Thomas Osborn,	University of Wisconsin
Joseph Ottobre	Ohio State University
Melanie Palmer	Oklahoma State University
John Parrish	University of Wisconsin
Alice N. Pell	Cornell University
Alan E. Pepper	Texas A&M University
T. Kaye Peterman	Wellesley College
James Petitte	North Carolina State University
Mason Pharr	North Carolina State University
Leland (Sandy) Pierson III,	University of Arizona
Oscar A. Pike	Brigham Young University
Erika Plisetskaya	University of Washington
Steven E. Poet	University of Georgia
Greg Pompelli	University of Tennessee
Tom E. Porter	University of Maryland
Matt Powell	University of Idaho
Stephen Quarles	University of California
Francis Quetier	GENOSCOPE
LeBris S. Quinn	Veteran's Puget Sound
Susan Quirk	Cornell University
Arthur G. Rand	University of Rhode Island
William R. Raun	Oklahoma State University
Katherine Reichelderfer Smith	USDA-ERS
Mark L. Richter	University of Kansas
Donald C. Robertson	University of Idaho
George K. Roderick	University of Hawaii
Kenton R. Rodgers	North Dakota State University
Wilson K. Rumbeiha	Michigan State University
Sabine J. Rundle	Western Carolina University
Matt A. Sanderson	USDA-ARS
Vicky Van Santen	Auburn University
Stephen G. Sapp	Iowa State University
Takuji Sasaki	Japanese Rice Genome Program
Thomas J. Sauer	University of Arkansas
James L. Seale Jr.	University of Florida
Jeffrey R. Seemann	University of Nevada
Magdi Selim	Louisiana State University
Donald W. Schaffner	Rutgers University
Floyd Schanbacher	Ohio State University
Christopher L. Schardl	University of Kentucky
K. Antoni Schat	Cornell University
Stanley S. Schnieder	University of North Carolina
James E. Schoelz	University of Missouri
Bruce Dale Schultz	Kansas State University
Mark W. Schwartz	University of California

325 Appendix B --Panel Members

Don Scott	University of Arkansas
Thomas D. Sharkey	University of Wisconsin
Kalidas Shetty	University of Massachusetts
Dale E. Shuster	Schering-Plough Animal Health Corporation
Marion Faye Simon	Kentucky State University
Thomas R. Slezak	Lawrence Livermore National Laboratory
Bruno W. Sobral	National Center Genome Research
David Sotomayor	University of Puerto Rico
James N. Spain	University of Missouri
Henry G. Spratt Jr.	University of Tennessee
Dina St. Clair	University of California
James Steele	University of Wisconsin
Donald Steinkraus	University of Arkansas
Bert E. Stromberg	University of Minnesota
Krishna V. Subbarao	University California
Joseph H. Sullivan	University of Maryland
George W. Sundin	Texas A&M University
Anthony Svejcar	USDA-ARS
Anne W. Sylvester	University of Idaho
Satoshi Tabata	Kazusa DNA Research Institute
Lincoln Taiz	University of California
Bernard Y. Tao	Purdue University
Phillip I. Tarr	Children's Hospital and Medical Center
Ming Tien	Pennsylvania State University
Patricia Timper	USDA-ARS
Peter A. Tipton	University of Missouri
Eileen Lenore Thacker	Iowa State University
Dawn Thilmany	Colorado State University
Gary A. Thompson	University of Arizona
Benjamin Tracy	USDA-ARS
Robert Turgeon	Cornell University
Carroll P. Vance	USDA-ARS, University of Minnesota
Vickie B. Vance	University of South Carolina
Mary E. Van Elswyk	Omega Tech Inc.
David W. Valentine	University of Alaska
Elizabeth Vierling	University of Arizona
Thomas I. Wahl	Washington State University
Elsbeth Walker	University of Massachusetts
Xuemin Wang	Kansas State University
Joseph J. Warthesen	University of Minnesota
Eric J. Weber	USEPA-ERD University of Wisconsin
Norman Weeden	Cornell University
Bart Weimer	Utah State University
Donald E. Weller	Smithsonian Environmental Research Center
Carol T. West	University of Florida

Janice K. Wiedenbeck	University of Kentucky
Lois Schertz Willett	Cornell University
Claire Williams	Texas A&M University
Kristina M. Williams	FDA-CFSAN
Nora E. Wineland	USDA-APHIS-VS
Michael Wolcott	Washington State University
Shang-Tian Yang	Ohio State University
Nevin Young	University of Minnesota
Zhao-bang Zeng	North Carolina State University
Maija Zile	Michigan State University
David J. Zimet	University of Florida
Elizabeth A. Zimmer	Smithsonian Institution

INDEX OF INVESTIGATORS

Abbott, E.A. 184
Abdelrahman, K.A. 246
Abrahamsen, M.S. 80, 90
Ackermann, M.R. 87
Adams, L.G. 51
Adams, M.B. 9, 222
Adaska, J. 56
Addy, K. 34
Aggarwal, R. 185
Ahearn, M.C. 221, 241
Ainsworth, A.J. 245
Alexander, L.J. 80
Allen, J.P. 168
Allen, R. 19
Alley, M.M. 222
Althouse, G.C. 62, 288
Alwang, J.R. 192
Amador, J.A. 259
Amasino, R.M. 145
Aminov, R.I. 72
Ammons, J.T. 34
Anastasio, C. 21
Anderson, A.J. 127
Andow, D.A. 122
Andrew, S.M. 232
Andrews, P.K. 223
Angirasa, A.K. 191
Anthony, N. 83
Anthony, R.V. 61
Antolin, M.F. 116
Apgar, G.A. 239
Arimoto, R. 254
Arjmandi, B.H. 45
Attie, A.D. 75
Atwill, E.R. 56
Aubert, D. 147
Auer C. 232
Babcock, B. 182
Backer, L. 57
Badinga, L. 61, 285
Baer, D.J. 41, 233
Bagnell, C. A. 65
Baker, D.C. 202
Baker, S.H. 54, 281
Bammel, B. 237
Banz, W.J. 239
Barbehenn, R.V. 121
Barbercheck, M.E. 222
Barletta, R.G. 94
Barnes, D.W. 78
Barnes, H.J. 93
Barton, M.K. 155
Baum, T.J. 118
Bautista, R.C. 195, 227
Bavister, B.D. 68
Baybutt, R.C. 42
Beadle, R.E. 89
Beale, S.I. 172
Beall, F. C. 211
Beard, J.L. 46
Beattie, G.A. 105
Beavis, W.D. 77, 144
Becker, J.M. 165
Beghin, J.C. 183
Beitz, D.C. 70, 86
BeMiller, J.N. 198
Benda, L. 35, 270
Bendich, A.J. 154
Benfield, D.A. 98, 304
Bengtson, D.A. 74, 260
Benito, M. 309
Berardinelli, J.G. 65, 249
Beremand, P.D. 202
Berenbaum, M.R 118
Berger, P.H. 106, 238
Berger, T. 60
Berlocher, S.H. 118
Bernal, J.S. 137, 295
Besser, T.E. 58
Beuchat, L. R. 48
Beverly, J.L. 71
Bhadula, S.K. 19, 265
Bhattacharya, M. 208
Bialowski, S. 26
Billington, S.J. 83
Biondini, M.E. 9
Birchler, J.A. 150
Bird, D.M. 123
Black, S.J. 64
Blackmer, A.M. 32
Blackmer, J.L. 133
Blair, J.M. 7
Blaner, W.S. 44
Blaschek, H.P. 206, 301
Blieszner, R. 193
Blissard, G.W. 124
Blocker, D.E. 255
Bloom, S.E. 32
Bloomquist, J.R. 74
Bohach, C.H. 49, 58
Bohach, G.A. 88, 238
Bohnert, H.J. 168, 298
Bohnhoff, D. R. 216
Bolker, J.A. 253
Bond, B. 10
Boone, R. 5, 226
Boor, K.J. 52, 292
Booth, S.L. 43
Bordovsky, J.P. 35
Botsford, J.L. 254
Bottje, W.G. 83
Boudreau, M.A. 136, 250
Bourquin, L.D. 199
Bowen, R.A. 61
Bowes, G. 13
Bowser, P.R. 95
Boyle, K.J. 221, 241
Boyle, S.M. 99
Braden, T.D. 70
Bradley, T.M. 74
Brandenberger, L. 57
Braun, E.L. 153, 281
Bray, T.E. 84
Breidt, F. 51
Brewer, M.J. 273
Brigham, L.B. 103
Britt, A.B. 140
Brogden, K.A. 86, 300
Bronson, C.R. 106
Bronson, K.F. 35
Brown, C.W. 54, 259
Brown, J. 31
Brown, K.M. 19
Brown, M.B. 85
Brown, W.C. 100
Brownie, C. 222
Browning Jr., R. 265
Brusseau, M.L. 28
Bryan, W. 223
Buell, C.R. 309
Bullerman, L.B. 52
Bullesbach, E.E. 263
Bultman, T.L. 122, 243
Bungay, H.R. 209, 255
Burandt, C.L. 208, 246
Burgess, D. 248
Burken, J.G. 244
Burnett, K.G. 97, 263
Burnett, L.E. 97, 263
Burns, R.T. 34
Busby, M. R. 266
Bushway, A.A. 243
Bushway, R.J. 241
Butler, J. 87
Buxton, D.F. 70
Bytnerowicz, A. 12

Cakmak, M. 210
Camire, M. 242
Carlson, J. 189
Carlson, J.J. 39, 276
Carlson, R.W. 174
Carriere, Y. 114
Carson, W.P. 9
Carter, C.A. 181
Carter, L. 237
Casavant, T.C. 77
Casey, J.W. 95
Cassida, K.A. 228
Castelo, M. 52
Caton, B.P. 129
Cavalieri, R. 137
Chakkalakal D. 45
Chandler, V.L. 146
Chang, K.O. 96
Chapman, K.D. 165
Chase, C.C. 264
Chavas, J.P. 187
Chen, F-C. 195
Chen, H. 25
Chen, L. 147
Chen, S. 223
Chen, W. 28, 230
Cheng, H.H. 79
Cheng, Z.M. 109, 252
Chescheir, G.M. 33
Chetelat, R.T. 140
Chiang, V.L. 213
Chinchar, V.G. 92
Chollet, R. 168, 298
Chory, J. 146
Christopher, D.A. 169, 235
Cirmo, C.P. 32, 256
Clagett-Dame, M. 46
Clark, T.M. 119, 288
Clarke, C.R. 96
Clay, C.M. 61
Clegg, M.T. 149, 229, 259
Clifford, A.J 40
Clinton, B.D. 10
Close, T.J. 144
Clouse, S.D. 163
Coble, H.D. 131
Cole, D.J. 33
Coleman, G. D. 213
Collins, J.C. 85
Collodi, P. 78
Comai, L. 154
Conklin, P.L. 18
Connolly, E.L. 18, 280
Cook, C.C. 189
Cooperband, L. 27, 296
Corredig, M. 197
Costa, J.T. 123, 291
Cotter, P.A. 84
Coupal, R. 193
Cox, T.L. 187
Crandall, A.D. 52, 293
Crawford, R. 212, 216, 237
Creamer, N.G. 222
Crofts, A.R. 170
Croteau, R.B. 127
Crull, S.R. 189
Culver, J.N. 107
Cumming, J.R 273
Curtiss III, R. 91
Cushman, J.C. 18
Czarnecka-Verner, E. 13
Czuprynski, C.J. 94
Damodaran, S. 203
Dandekar, A.M. 205
Dangl, J.L. 152
Daniels, W.B. 47
Davies, J.P. 14, 287
Davin, L.B. 215
Davis, J.G. 32
Davis, J.S. 63
Davis, W.C. 100
Dayton, W.R. 73
de Gorter, H. 185
de Jong, P.J. 76
Dean, D.R. 177
Decker, E.A. 199
Delany, M.E. 76
DeMartini, J.C. 85
Denison, R.F. 174
Dennehy, T. 114
Dennis, D.F. 221
DeWald, D.B. 166, 269
Dickstein, R 177
Diehle, J. 261
Diers, B.W. 142
Dighton, J. 24
DiMario, J.X. 71, 287
Dimmel, D. R. 211
Ding, B. 111, 257
Dobrinski, I. 81, 294
Dobson, S.L. 120, 289
Dodgson, J.B. 79
Donis, O.R. 94
Dooley, F.J. 220
Doster, A.R. 95
Downie, D.A. 115, 276
Drake, M.A. 50, 291
Drost, D.T. 270
Drummond, F.A. 8
Duby, R.T. 64
Dudley, L.M. 26
Dueker, S.R. 40
Duff, S. F. 214, 294
Dunlap, J.R. 267
Dute, R.R. 226
Duvall, M.R. 264
Dvorak, J. 147
Earle, E.D. 125
Ebbole, D.J. 111
Edwards, J.L. 267
Ehleringer, J.R. 10
Eifert, J. 54, 295
Elekonich, M.M. 119, 278
Ellis, R.W. 237
Emerich, D.W. 175
English, B.C. 187
English-Loeb, G.M. 124
Epperson, B.W. 263
Ernst, C.W. 79
Essenberg, R.C. 125
Evans, E.W. 126
Faaberg, K.S. 90, 290
Falck-Zepeda, J.B. 180
Falk, B.W. 104
Fang, C. 182
Fang, F.C. 51
Farrah, S. 30
Fausti, S.W. 263
Faustman, C. 196, 231
Fay, P.A. 7
Feder, J.L. 119
Fennell, A. 264
Ferro, D.N. 120
Ficht, T.A. 98
Findeis, J.L. 192
Findies, J.L. 191
First, N.L. 81
Fischer, A.J. 129
Fissore, R.A. 64
Fitzsimmons, K.M. 219
Flebbe, P.A. 221
Foin, T.C. 129
Foley, D. 47, 228
Ford, C. 197
Ford, R.G. 23
Forsberg, L.S. 174
Fox, J.A. 184
Foyer, C. 168, 298
Francis, D.H. 98
Frank, H.A. 169
Frank, J.F. 48
Frankel, D.J. 241
Frankenberger, W.T., Jr. 29
Freeland, R.S. 34
Frisch. D. 262
Fuerstenberg, S.I. 162, 278
Fulton, J.R. 220
Gadoury, D.M. 124
Gallie, D.R. 148
Gamble, M.V. 45, 280
Ganguly, R. 126

Gascho, G.J. 220
Gehrt, K.C. 181
Gelberg, H.B. 88
George, D.B. 35, 266
German, T.L. 113
Gianola, D. 82
Giblin, T.L. 29
Gibson, K.D. 129
Gibson, S.I. 267
Gilbertson, R.L. 148
Gilliam, F.S. 272
Gilliam, J.W. 33
Gimenez, D. 222
Giner, J.L. 256
Ginther, O.J. 69
Giroux, M.J. 152, 248, 291
Glasser, W.G. 215
Glenn, E.P. 219
Godkin, J.D. 67
Goetz, F.W. 63
Golbeck, J.H. 172, 304
Gold, A.J. 34
Gold, S.E. 105
Goldberg, B.B. 54, 259
Goldstein, W.A. 224
Goll, D.E. 72, 298
Gonzalez, C.F. 111
Gonzalez-Meler, M.A. 171
Goodenough, U.W. 151
Goodhue, R.E 181
Goodwin, Jr., H.L. 219, 227
Gopinath, M. 186
Gorres, J.H. 259
Gould, B.W. 188
Gould, J.R. 133
Goulden, M. L. 5
Grady, K.A. 265
Graef, G.L. 142
Granados, R.R. 124
Grasso, D. 29
Grazul-Bilska, A.T. 252
Greenwood, M.S. 161, 242
Grimes, H.D. 178
Griswold, K.E. 239
Griswold, M.D. 68, 305
Grizzle, J.M. 267
Groffman P.M. 34
Groom, L. H. 213
Gross, D.J. 64
Grover, T.A. 269
Grummer, R.R. 75
Grusak, M.A. 20, 43, 304
Grygiel, C.E. 9
Guerinot, M.L. 176
Guillard, K. 30, 231
Gulig, P.A. 47
Gundersen-Rindal, D. . . . 121, 289
Gunner, M. 172, 256
Gunter, S.A. 228
Guo, A. 206, 240
Guo, Y.H. 109, 252
Gurley, W. B. 13
Hagler, J.R. 133
Haile, F.J. 122
Hales, B.J. 175
Hama, H. 166, 269
Hambidge, K.M. 40
Hancock, D.D. 58
Handler, A.M. 117
Hanks, L.M. 116, 134
Hanna, M.A. 52
Hanna, W.W. 141
Hansen, P.J. 62, 299
Hanson, G.D. 191
Hanson, L.A. 91, 245
Hardy, M.E. 248
Hardy, R.W. 238
Hargrove, M.S. 170, 287
Harkema, J.R. 89
Harmon, M. 25
Harrison, J. 223
Hart, R. 57
Hartel, R.W. 204
Hathaway, M.R. 73
Hatley, O.E. 191
Havel, P. J 40
Hawes, M.C. 14, 103
Hawk J.A. 234
He, S.Y. 108
Hedgecock, D. 76
Helm, R. F. 215
Henderson, D.A. 186, 191
Henneberry, T.J. 133
Heppert, J.A. 207
Herrin, D.L. 154
Hess, B.W. 273
Highlander, S.K. 99
Higley, L.G. 122
Hill, J.E. 129
Hill, V.R. 33
Hillers, V. 53
Hirano, S.S. 113
Hiremath, S.T. 24
Hixon, D. L. 69, 274
Ho, M. 88
Hoelmer, K.A. 135, 300
Hoeschele, I. 81
Hoffman, B.M. 174
Hoffman, R.M. 232
Holaday, A.S. 19
Holl, K. D. 6, 229
Hollick, J.B. 147
Holmén, B.A. 21
Holmes, R.P. 44, 303
Holt, J. 129
Holway, D. 116
Hook, P.B. 130
Hoover, D.G. 47
Horne, S.M. 251
Horner, H.T. 106
Horohov, D.W. 89
Horton, T.R. 25
Horwath, W.R. 22
Hotchkiss, J.A. 89
Howell, C. 137
Hsiao,T.C. 168
Hsieh, Y-H.P. 195
Huang, A.H.C. 157
Huang, A.S. 14
Huang, J.K. 240
Huber, K.C. 238
Huber, S.C. 163
Hughes, M.G. 222
Hultin, H.O. 199
Hunt, A.G. 150
Hunt, C.W. 49
Husband, T.P. 259
Hussey, R.S. 117
Hutchison, K.W. 161, 242
Hutchison, W.D. 122
Hyde, G.M. 203
Ibarra, J. 195
Inglis, D.G. 245
Inman, K. 193
Innes, R.W. 143
Inzana, T.J. 99
Islam, S. 24
Jackman, A.P. 205
Jackson, C.G. 133
Jackson, D. 163
James, M.G. 159
Jaykus, L. 57
Jeffery, E.H. 198
Jeffrey, J. 56
Jensen, L. 192
Jerry, D.J. 78
Johnson, A.L. 63
Johnson, B.J. 263
Johnson, G. C. 211
Johnson, K.B. 136
Johnson, M. 195
Jones, A.M. 163, 303
Jones, J.B. 8, 254, 292
Jones, R.H. 10
Joshi, C.P. 213

Jost, B.H. 83
Junk, V.W. 189
Jutila, M.A. 92, 248
Kalaitzandonakes, N. 185
Kalapathy, U. 227
Kaneene, J.B. 57
Kao, C.C. 106
Kao, T.H. 164
Kaplan, D.L. 207
Kapros, T. 151, 244, 290
Karowe, D.N. 121
Kask, S.B. 251
Kathariou, S. 48
Kattesh, H.G. 267
Kaufman, L.S. 160
Keen, N.T. 104, 299
Keim, N.L. 40
Kelleher, S.D. 199
Kells, S. 134
Kempf, S.C. 226
Kendall, D.A. 158, 231
Kendall, P. 53
Kenerley, C. 137
Kenney, M. 219
Kerfeld, C.A. 169
Khan, I. A. 208, 246
Kianian, S.F. 252
Kielland, K. 5, 226
Kiely, D. 249
Kieszak, S. 57
Kilkenny, M. 190
King, B.A. 31, 237
King, G.M. 23, 242
King, M. 89
Kirby, J. 83
Kirk, A.A. 135, 300
Kirk, J. 56
Kitchen, N.R. 32
Kittredge, D.B. 221
Kjolhede, C. 45
Klaenhammer, T. R. 200, 303
Klei, T.R. 89
Klein, R.R. 141
Kleinhofs, A. 144
Klemme, R. 224
Klessig, D.F. 109
Kloepper, J.W. 133
Knapp, A.K. 7
Knapp, J.R. 75, 271
Knapp, S.J. 140
Kocher, T. 253
Koenning, S. 222
Kohorn, B.D. 171
Koide, R. 24
Konkel, M.E. 55
Koohmaraie, M. 200
Kotcon, J.B. 223
Krannich, R.S. 192
Kranz, R.G. 176, 208
Krebs, N.F. 40
Kresovich, S. 142
Krishnan, P.G. 265
Kropf, D.L. 166, 268
Krutovskii, K.V. 144
Kuhlenschmidt, M.S. 88
Labate, J.A. 142
Lakritz, J. 90, 290
Lam, E. 109
Lambert, K.N. 115
Lamberti, G.A. 7
Lamkey, K.R. 142
Lan, Q. 236
Landis, D.A. 135
Laporte, M.M. 170, 277
Lascano, R.J. 35
Lathrop, R. 190
Lauzon, C. 115, 228
Lawrence, M.L. 91, 246
Layton, A.C. 34
Leach, J.E. 143
Leach, J.L. 104, 299
Lee, D. 182
Legaspi, J.C. 137, 295
Lei, K.Y. 38
Lemieux B.R. 234
Lemley, A.T. 32
Letcher, S.. . . . . 54, 259, 260
Leung, H. 143
Lewbart, G.A. 93
Lewin, H.A. 77
Lewis, D.S. 70
Lewis, N. G. 215
Libby, S.J. 51
Libby, W.L. 221, 241
Lichtenberg, E. 185
Lill, J.T. 122
Lin, C. 157
Lin, X. 309
Lindow, S.E. 103, 299
Lini, A. 11, 271
Liscum, E. 162
Liu, B. 156, 284
Lo, Y.M. 196, 234, 285
Logan, B. 19
Loo, D.D.F. 156
Lookhart, G. 199
Loria, R. 110
Lory, J.A. 32
Lotz, S. 181
Louws, F.J. 222
Love, B.C. 58
Luan, S. 13
Lucas, W.J. 148
Lukaszewski, A.J. . . 149, 229, 259
Lukat-Rodgers, G.S. 251
Luloff, A.E. 191
Lussenhop, J. 24
Lynch, J.P. 19
Ma, P.W.K. 245
MacFall, J.S. 131
Machaty, Z. 64
Mackie, R.I. 72
Mackill, D.J. 140
MacLachlan, N.J. 84
MacRitchie, F. 199
Madigan, M.T. 208
Magnuson, B.A. 49
Maier, D.E. 134, 220
Malayer, J.R. 257
Mallikarjunan, P. 54, 295
Marcotte, Jr., W.R. 260
Marquis, R.J. 122
Marra, M.C. 222
Martin, G.B. 152
Mason, G.L. 84
Mason, H. S. 205
Mason, L.J. 134
Mason, P.W. 95
Matthews, B.F. 121
Matuana, L. M. 214, 243, 289
Maurer, J. 50, 56
May, J.D. 272
Mayama, S. 104, 299
McCarty, D.R 14
McClements, D.J. 199
McCombie, R.W. 309
McCray Jr., P.B. 87
McDonald, K.A. 205
McDonald, L. 223
McDowell, W.H. 9, 253
McGrane, M.M. 41
McHale, N.A. 158, 230
McHugh, J.V. 117
McInnes, K.J. 25
McIntosh, M.K. 250
McLeod, D 193
McWilliams, S.R. 260
Medeiros, L. 53
Meiners, S.J. 131, 280
Menalled, F.D. 135
Michelmore, R. 140
Milgroom, M.G. 110
Millam, J.R. 60
Millar, J.G. 116
Miller, B.E. 26

Miller, C. 250
Miller, C.L. 8, 279
Miller, D.J. 62
Miller, D.M. 21
Miller, J.B. 72
Miller, J.F. 84
Miller, M.E. 226
Miller, N. J. 190, 286
Miller, N.W. 92
Miller, O.K. 10
Miller, W.L. 65
Mills, B.F. 192
Mitchell, J.P. 220
Mitchell, R.J. 10
Miyasaka, S.C. 14
Moe, C.L. 57
Mohamed, E.A. 246
Mohamed, R. 144
Mohanty, S. . . . 125, 183, 286, 293
Molin, W.T. 131
Molina, R. 25
Moll, D. 57
Molofsky, J. 272
Monks, D.W. 222
Monteiro-Riviere, N.A. 93
Montville, T.J. 52
Moore, D.A. 57
Moore, J.N. 85
Moore, M.M. 99, 282
Moraes, R. M. 208, 246
Morgan, R.W. 85
Morishita, T.Y. 58
Morley, P.S. 58, 84
Morra, M.J. 31
Morris, T.F. 30, 231
Morrison, M. 73
Morse, P.D. 49
Mosier, A.R. 35
Moss, C.B. 182
Moss, G. E. 69, 274
Moss, S.B. 66
Moss, S.M. 70, 235, 285
Mottinger, J.P. 153
Mou, P. 10
Mueller, J.P. 222
Mulchandani, A. 29
Mullen, M.D. 34
Muller, C.D. 191
Mulvaney, S.J. 201
Murphy, G.L. 96
Murphy, J.F. 133
Myers, A.M. 106, 159
Myles, D.G. 66, 299
Nadeau, J.A. 164
Nair, M.G. 199
Neill, J. 86
Nelson, E.B. 136
Newton, G.L. 220
Ni, X. 122
Nikolau, B.J. 150
Nilsen, E.T. 10
Nishimoto, R.K. 130, 236
Noga, E.J. 93
Norton, J.M. 26
Novotny, R. 41, 235
Nute, D.E. 221
O'Brian, M.R. 176
Okimoto, R. 83
Oldenburg, D.J. 154
Oliver, D.J. 15
Olson B.H. 28
Olson, B.E. 130
Opp, S. 115, 228
Or, D. 26
Orndorff, P.E. 93
Osorio, F. 95
Ostoja-Starzewski, M. 211
Oudemans, P. 222
Outlaw, Jr.,W.H. 14
Ozias-Akins, P. 141
Pack, R.T. 35, 270
Padgett, P.E. 12
Paine, T.D. 116
Palmer, G.H. 100
Panaccione, D.G. 273
Parker, D.B. 268
Parker, V. T. 6
Parkin, K.L. 203
Parks, B.M. 167
Parks, P. 190
Parveen, S. 30
Pascual, D.W. 93, 248
Paszczynski, A. 212, 237
Patterson, P.M. 180
Payne, G.A. 51
Pecherer, R.M. 144
Perry, K.L. 107
Perryman, L.E. 94
Peterman, T.K. 161, 240
Peterson, D.G. 141, 277
Peterson, H.H. 186
Petrovic, Z.S. 206, 240
Pick, D.H. 181
Pickard, B.G. 17
Pickart, C.M. 73, 301
Pignatello, J.J. 22
Pikaard, C.S. 151
Pivarnik, P. 54, 259
Podila, G.K. 24
Pomp, D. 77
Ponder, G. 249
Porter, A.H. 120
Porter, S.D. 134
Portier, K. 30
Pote, L. 92
Powers, R.F. 22
Prakash, A. 47, 228
Prather, R.S. 77
Pring, D.R. 141
Quackenbush, S.L. 95
Quimby, P.C. 135, 300
Quinn, M.A. 11
Quinn, M.T. 248
Quisenberry, S. 122
Radin, J. 88
Ragauskas, A. J. 212
Ralph, J. 217
Raman, D.R. 34
Rand, A.G. 54, 259, 260
Randall, S.K. 16
Rao, A.L.N. 105
Rasmussen, K.M. 45
Rauscher, H.M. 221
Ray, C. 30, 286
Ray, D.E. 187
Rboy, V. 40
Rea, P.A. 165
Redmer, D.A. 252
Reed, H.C. 258
Reed, S.M. 96
Reed, W.A. 68, 270
Reganold, J.P. 223
Reilly, P. J. 197
Reiter W.D. 232
Renner, K.A. 135
Reuhs, B.L. 174
Reynolds, J.E. 189
Reynolds, L.P. 252
Rhodes, S.J. 78
Richards, T.J. 180
Riddiford, L.M. 126, 127, 305
Ridpath, J. 86
Rikihisa, Y. 96
Riley, M.B. 262
Ristic, Z 19, 265
Rizvi, S.S.H. 201
Roberto, K.A. 193
Roberts, D.W. 127
Roberts, G.P. 178
Robinson, N.E. 89
Robl, J.M. 78
Robson, R.M. 71
Roche, D. 141
Rodermel, S.R. 118
Rodgers, K.R. 251

Rodriguez, M.L. 184
Rodriguez-Kabana, R. 133
Rogers, S.D. 272
Romero-Severson, J. 119
Ronald, P.C. 140
Rosowsky, D.V. 214, 261
Ross, D.S. 11, 271
Ross, K.G. 117
Roth, J.A. 86, 300
Rothschild, M.F. 77
Roudebush, W.E. 261
Roux, K.H. 197
Rowland, R.R. 98, 264
Rowlett, R.S. 255
Rozman, S.L. 246
Ruess, R. 5, 226
Rufty, T.W. 131
Rusling J.F. 169
Russell, A.E. 7, 277
Russell, R.M. 43
Russell, S.D. 164
Sachs, C.E. 191
Sack, F.D. 164
Sadof, C.S. 134
Sahlu, T. 257
Saif, L. 88, 96
Salmon, M. 56
Saltzman, E. 43
Sanderson, M.W. 50
Sanford, Jr., R.L. 230
Sanger, J.M. 97
Sankula, S. 130, 234
Santo, G.S. 31
Sapp, S.G. 184
Sargeant, J.M. 50
Sartin, J.L. 70
Sasahara, K.C. 53, 281
Sathe, S.K. 197
Sauer, J.R. 125
Sauer, T.J. 219, 227
Saville, W.J. 58
Sawyer, H.R. 61
Sayler, G.S. 34
Scanlon, M.J. 159
Schäfer, L 21
Scharf, P.C. 32
Schifferli, D.M. 97
Schimpf, M.E. 237
Schmidt, C.J. 77, 233
Schmidt, R.J. 149
Schmits, A. 182
Schmoldt, D.L. 221
Schnable, P.S. 150
Schoeb, T.R. 85
Scholthof, H.B. 112
Scholthof, K.B.G. 112
Schook, L.B. 80, 302
Schooley, D.A. 123
Schrick, F.N. 266
Schuerman, P.L. 162, 279
Schuler, M.A. 118
Schupp, E.W. 132
Schwarz, J.M. 39, 284
Scott, M. P. 205
Scott, T.M. 258
Scow, K.M. 22
Sears, R. 199
Seem, R.C. 124
Segee, B. 241
Selinger, D.A. 146
Shackel, K.A. 174
Shackelford, S.D. 200
Shelby, K.S. 120
Shelton, A.M. 125
Sherman, J.D. 249
Sherwood, J.E. 108
Shih, M.C. 16
Shiller, A.M. 247
Shim, S. 181
Shoemaker, D.D. 117
Shultz, J. 195
Sibley, L.D. 91
Siebenmorgen, T.J. 195, 227
Sieburth, L.E 269
Siedow, J.N. 171
Sischo, W.M. 56
Skaggs, R.W. 33
Skinner, J.S. 144
Skonberg, D. 242
Slaymaker, D.H. 110, 276
Smart, L.B. 256
Smets, B.F. 29
Smith, M.O. 267
Smith, M.T. 189
Snyder, W.E. 128, 282
Soares, M.B. 77
Sobsey, M.D. 33, 57
Soller, M. 79
Soltys, J. 248
Songer, J.G. 83
Sordillo, L.M. 46
Soucek, M.D. 209
Southard, R.J. 220
Southworth, D. 258
Spalding, E.P. 167
Spalding, M.H. 15
Sparks, D.L. 23
Specker, J.L. 74, 260
Splitter, G.A. 101
Spratt, H. 35, 266
Spreitzer, R.J. 171
Stacey, G. 165, 177
Stahl, D. C. 216
Staiger, C.J. 160
Stark, J.C. 31, 237
Stark, J.M. 26
Steadham, E.M. 87
Stearman, G.K. 35, 266
Steber, C.M. 166, 295
Steiner, R.L. 189
Sterling, T.M. 131
Stevens, T.H. 221
Stevenson, D.M. 210, 292
Stockle, C.O. 223
Stockwell, V.O. 136
Stoecker, B.J. 45
Stokes, J.R. 186
Stolt M. 34
Storm, C. 87, 301
Strand, M.R. 120
Strasburg, G.M. 199
Stratton, D. 271
Strauss, S.H. 25, 144
Stubbs, C.S. 8
Sudduth, K.A. 32
Sultan S.E. 232
Summers, A. 56
Sun, T.P. 163
Sundin, G.W. 112
Sung, Z.R. 147
Suntermann, C.A. 226
Suppes, G.J. 207
Sutovsky, P. 66, 293
Swain, S.D. 247
Swanson, B. 137
Szymanski, D.B. 160, 288
Tabashnik, B. 114
Tack, B.F. 87
Talbert, L.E. 249
Tang J. 137
Tang, G 43
Tao, Y. 195
Tarboton, D.G. 35, 270
Taren, D.L. 38, 298
Taylor, J.W. 22
Tchikindas, M. 52
Teisl, M.F. 184, 241
Temple, L. 93
Teppen, B.J. 21
Thiel, T. 17
Thomas, P. 67
Thomas, T.L. 202
Thomashow, M.F. 17
Thompson, D.B. 201
Thompson, G.A. 114, 166

Thon, M.R. 107, 278
Thurston, R. J. 67, 262
Tickamyer, A.R. 186, 191
Tiffin, P. 148, 279
Tilton, J.E. 252
Tobin, E. 157
Tomek, W.G. 186
Traxler, G. 180
Trick, H.N. 143
Trumbore, S. E. 5
Tsao, G.T. 206
Tsuyumu, S. 104, 299
Tuggle, C.K. 77
Tulloch, D. 190
Tyler, S. C. 5
Ullman, D.E. 113
Umbach, A.L. 171
Umen, J.G. 151
Upper, C.D. 113
Van Driesche, R.G. 135
Vance, C.P. 175, 302
Vanderhoek, J.Y. 41, 233
VanEtten, H.D. 21, 103
VanGessel, M.J. 130, 234
Varsa, E.C. 239
Vellidis, G. 220
Venkitanarayanan, K. 232
Venta, P.J. 79
Vetelino, J.F. 243
Vickers, N.J. 268
Vieille, C. 207
Vierling, E. 12
Vierstra, R.D. 73, 301
Villet, R. 135, 300
Vinopal, R.T. 29
Vitousek, P. M. 6
vonBodman, S. 232
Vulpe, C. 39, 284
Wagger, M.G. 222
Wagner, D. 8, 254, 292
Walcheck, B. 90
Walker, E.L. 16
Walker, M. 254
Wall, R.W. 31, 237
Wallig M.A. 198
Walling, L.L. 149, 229
Walton, J.D. 108
Wander, R.C. 44
Wang, C. 265
Wang, H.H. 187, 296
Wang, J. 29
Wang, K. 205
Wang, P. 124
Wang, Y.-J. 195, 227
Waters, L.C. 126
Watkins, C.B. 200
Weathers, L. 35, 266
Weaver, R.D. 186
Webb, B.A. 120
Webb, K.E. Jr. 74
Wei, N. 158
Weigel, D. 146
Weigel, R.M. 57
Welsh, J. 42
Wen, L. 240
Wen, Z. 195
Wessling-Resnick, M. 43
West, T. P. 264
Wheeler, T.L. 200
White, A.M. 78, 278
White, B.A. 57
White, D. 56
White, D.G. 50, 56
White, J.A. 186, 191
White, K.L. 68
White, M. 248
White, M.E. 73
White, W.S. 42
Whitfield, K. 46
Whitworth, T. M. 32
Wicker, L. 197
Wideman Jr., R.F. 83
Wiedmann, M. 52, 292
Wilkinson, B.J. 49
Wilkinson, H.H. 153, 294
Willet, K.D. 219, 227
Williams, C.G. 145
Williams, D.L. 268
Williams, J.C. 168
Willits, F.K. 191
Wilson, B.A. 88
Wilson, D 56
Wilson, R. 309
Wing, R.A. 144, 309
Winters, T.A. 239
Wise, R.P. 142, 144
Wittum, T.E. 58, 84
Wolfe, R. W. 216
Woloshuk, C.P. 49, 134
Wolpert, T.J. 111
Womack, J.E. 77
Wong, E.A. 74
Wood, R.J. 43
Woods, S.A. 8
Wooten, M.W. 226
Wossing, A. 222
Wraith, J.M. 130
Wu, L. 213
Wu, Q. 212
Wurtele, E.S. 15, 300
Xiang, C. 15
Yablonka-Reuveni, Z. 75
Yamamoto, H.Y. 236
Yang, S.T. 209
Ye, Z.H. 159
Yeates, T.O. 169
Yi Li 232
Yocum, C.F. 171
Yoder, R.E. 34
Young, J.W. 86
Young, T.M. 21
Yucheng, F. 226
Zabowski, D. 26
Zeevaart, J.A.D. 161
Zehnder, G.W 133
Zeikus, J.G. 207
Zhang, D. 180
Zhang, H. 79
Zhang, Q. 58
Zhao, S. 50
Zimmermann, N.G. 31, 302
Zuckermann, F. 95
Zuerner, R.L. 87, 301
Zydney, A.L. 196, 233