Plant Diseases National Program

National Program Overview: ARS Research is organized into 22 National Programs. These programs serve to bring coordination, communication and empowerment to the more than 1200 research projects carried out by ARS. The National Programs focus on the relevance, impact, and quality of ARS research. These National Programs are organized within four broad areas: Nutrition, Food Safety/Quality (NFSQ); Animal Production and Protection (APP); Natural Resources and Sustainable Agricultural Systems (NRSAS); and Crop Production and Protection (CPP). The Plant Diseases National Program (NP303) is part of CPP but interaction also occurs with researchers in APP, NRSAS and NFSQ. The annual budget is $66,593,891 and 158 scientists conduct research at 41 locations.

Each National Program conducts a planning workshop to focus the research program by learning the problems and needs of customers, stakeholders and partners. These workshops help ensure that our research programs are relevant to the concerns of our constituents. The Plant Diseases National Program Workshop was held in Beltsville, Maryland, October 1999. Approximately 70 customers and stakeholders and 50 ARS scientists attended. The participants identified the important disease problems facing their crops and commodities. Ongoing research in each problem area was discussed.

Based on input received at the 1999 planning workshop, research in the Plant Diseases National Program was organized into 5 component areas: **Identification and Classification of Pathogens**; **Biological Control**; **Cultural Control**; **Pathogen Biology, Genetic, Population Dynamic, Spread and Relationship with Hosts and Vectors**; and **Host Plant Resistance to Disease**. Teams of ARS Scientists worked with National Program Leaders to develop an Action Plan that provided the framework for ARS research over a five-year period. Each of the five components contained problem areas that required research. Groups of ARS scientists wrote Project Plans that described the research they would conduct, the anticipated products or information to be generated by the research, roles and responsibilities of ARS scientists and their cooperators, and timelines and milestones to measure progress toward achieving the goals of the research. These project plans were reviewed for scientific quality by a panel of experts in the field. ARS used input from the panel to revise and improve their planned research.

The final stage in the cycle of an ARS National Program involves assessment of research that has been conducted. This expert panel on which you are a member, is assembled to evaluate the impact of ARS research accomplished and to offer suggestions for future ARS research direction and emphasis. Input from this panel and from interested parties at a planning workshop to be held May 17-19, 2005, in Orlando, Florida, will be used to develop the framework for ARS research over the next five years.

Program Summary: The overall goal of the Plant Diseases National Program is to develop and improve ways to reduce crop losses caused by plant diseases. The program focuses on developing effective disease control strategies that are not environmentally harmful, do not threaten the safety of consumers, and are compatible with sustainable and

profitable crop production. The ARS program is conducted in cooperation with related research in other public and private institutions.

Plant diseases, caused by viruses, viroids, bacteria, phytoplasmas, fungi, and nematodes, result in economic losses in agriculture, landscape, and forest settings by reducing yields, lowering product quality or shelf-life, decreasing aesthetic or nutritional value, and, sometimes, by contaminating food and feed with toxic compounds. Control of plant diseases is essential to providing an adequate supply of food, feed, and fiber. Growers currently spend large sums to achieve partial control of pathogens that attack crops and other plants. Even then, crop and commodity losses caused by diseases cost billions of dollars each year. Reducing such losses has long been a high priority objective for agriculture and also for the Agricultural Research Service (ARS). Besides the obvious monetary benefits for producers and processors, successful plant health protection is important if we are to maintain and increase food supplies with minimum increases in land under cultivation. Additionally, knowledge and management of plant diseases of quarantine significance are vital, not only for protecting our domestic crops from foreign disease, but also for maintaining and expanding export markets for plants and plant products.

Strategies for the control of plant diseases include: planting resistant crop varieties; changing crop cultural practices or storage conditions to those less favorable for disease development; employing biological controls; applying chemical pesticides; and using integrated disease management (combining two or more of the above approaches). The ability to develop any of these strategies depends first on identifying the pathogen causing the disease, then learning how to interrupt its disease cycle. The more known about the genetic, biochemical, and physiological processes that operate in the host and pathogen as infection and disease progress, the more likely a control method can be devised. Understanding the ecology of pathogens (that is, how they survive, are dispersed, and otherwise interact with their environment) is also important. In addition, an understanding of the epidemiology or outbreak of disease and spread of pathogens is important for deciding which control actions are most effective.

As with all microorganisms, plant pathogens exhibit a remarkable ability to change and adapt. Newly discovered pathogens and more virulent strains of old pathogens continually arise and often overcome resistant crop varieties or can no longer be controlled by strategies and chemicals that were once effective. Continual research to develop new control methods is necessary to increase or even maintain current levels of crop production and commodity preservation. Further complicating the situation, public concern has grown in recent years regarding the use of synthetic chemicals to control diseases. This concern stems from the fear that such chemicals may contaminate food or accumulate in the soil and ground water (and so be introduced into the food chain). As a result, pressure has increased to develop nonchemical approaches to plant disease control. Typically, nonchemical controls do not exist, are less effective, or too costly. More research is needed to develop or improve nonchemical control methods and procedures.

Host plant resistance to plant diseases is probably the most desirable control strategy because it can be highly effective, is environmentally benign, and usually entails little or no additional expense to producers. To be most effective, such resistance should be durable, i.e., not readily overcome by mutations in pathogens that increase their virulence. This requires detailed knowledge of the pathogens that are present, their ability to change, and the nature of the resistance processes themselves.

For diseases where host plant resistance is unavailable or only somewhat effective, strategies are needed that integrate cultural, biological, and chemical control procedures. For example, different crop rotations or improved cultural practices can sometimes suppress development of pathogenic organisms while maintaining a high level of productivity. Biological control shows great potential for disease control, but has often been unreliable or only marginally effective. Additional research is needed to understand the interactions of microorganisms in the soil, plant parts, and on the mechanisms that biological control agents use to reduce or prevent the onset and development of disease. Finally, the appropriate use of chemicals will continue to be an important tool in the battle against plant diseases, especially where alternative controls are not effective. Additional research is needed to develop safer chemicals and to increase the efficiency of chemical applications.

Also required are rapid, reliable pathogen detection and identification procedures for accurate and timely disease diagnoses. Improved detection and identification procedures are also becoming more important as international trade of plant products increases and as trading partners seek to protect themselves from the introduction of unwanted diseases.

Recently developed biotechnology tools provide promising new approaches for achieving control of diseases. Genetically engineering plants for resistance to viral, bacterial, fungal, and nematode pathogens has shown some success, but more research is needed. Also, techniques have been developed that allow researchers to track specific resistance genes, facilitating breeding resistant varieties. Biotechnology offers the exciting possibility of developing disease resistance in plants that cannot be accomplished through conventional breeding procedures (for example, by introducing genes for resistance from unrelated species).

Additionally, Congress has appropriated funds to help solve specific production and marketing problems of the United States industry. ARS has decided that some of the funds will be used for agreements with State Agricultural Experiment Stations and similar research institutions. Proposals are solicited by the National Program Staff and are evaluated by ARS, state scientists and industry representatives. Criteria used to evaluate the proposals include: national priority of the research, scientific expertise of those involved in the project, funding requested, and probability of success in solving production and marketing problems.

Funds are maintained at ARS headquarters because the program covers several regions and national programs. Proposals funded are designed to demonstrate accomplishments

in key areas aligned to the objectives of the Plant Diseases National Program: key diagnostic tools for identification of pathogens, resistance genes to devastating diseases, understanding of pathogen biology, and epidemiology and vector relationships. Current projects funded by this process include: The Potato Research Program, The Small Fruit and Nursery Research Program, The Fusarium Head Blight Program, and The Floral and Nursery Research Initiative.

Identification and Classification of Pathogens Component

Background: Effective disease control depends on rapid and accurate identification of the pathogens involved so that appropriate control measures may be taken. Accurate identification of pathogens is also critical for making sound decisions regarding quarantines of imported and exported plant materials and commodities. Knowing how pathogens are related to each other can be helpful in suggesting possible control strategies. ARS research has significantly impacted science and the public because of accomplishments in identifying, characterizing, and classifying a broad spectrum of pathogens.

Structural and functional genomics of potato, strawberry, grapevine, jujube, walnut, bean, carrot, peach, cherry, pear, and almond pathogens: Plant diseases caused by vascular system-inhabiting wall-less bacteria (mollicutes, phytoplasmas and spiroplasmas) and walled bacteria are responsible for significant economic losses to U.S. and world agriculture. Accurate, rapid and sensitive technologies for detection and identification of these pathogens and their ecologies are vital prerequisites for decisions to formulate appropriate disease control measures and for intercepting pathogens under quarantine regulations. Knowledge gained from structural and functional genomics is making it possible to devise improved and novel means for genus-level, species-level, and virulent strain-level detection and identification of these pathogens, including invasive species. Comparative genomic studies will lead to identification of potential genes useful for detection and identification of the pathogens at various taxonomic levels. Genomic information and the new knowledge of bacterial pathogenesis will promote fundamental and applied research on disease control.

Floral and woody ornamental plant prokaryotes: Bacterial diseases caused by *Pseudomonas, Xanthomonas, Xylella, Ralstonia* and other genera often result in significant losses in the production and quality of ornamental crops, and are very difficult to control. Although *X. fastidiosa* has been known to cause leaf scorch diseases of woody ornamentals for 20 years, some basic questions still remain largely unanswered. These include the host range of *X. fastidiosa* in horticultural and alternative plants, and the genetic and pathogenic relationships among strains of *X. fastidiosa* isolated from economically important horticultural and agricultural hosts and from invasive alternate hosts in the environment. Recent surveys indicate that the disease is spreading and becoming more severe in important landscape trees such as oak, elm and sycamore in many states. Bacterial leaf scorch of oleander, a relatively new disease reported in 1999, is also an emerging problem in California, Arizona and Texas where oleander is used as a popular landscape plant for residential hedges, highway dividers, colorful accents and beach plantings. In order to effectively control bacterial leaf scorch diseases of landscape trees and shrubs, answers to the above questions are greatly needed.

Crown gall and deep bark canker (DBC) of nut trees (walnuts/almonds: Crown gall, caused by the bacterium *Agrobacterium tumefaciens*, is the most important disease of walnuts in California; there is no known effective control strategy. The disease is present in all walnut-growing regions of the state and can occur on 80% of the trees in a given

orchard causing significant yield losses, and sometimes tree death. Initial efforts are focusing on the design and development of robust detection methods for *Agrobacterium*. In the southern range of the walnut-growing region, DBC (*Brennaria rubrafaciens*) is eliminating the use of a walnut cultivar that once represented over 60% of the walnut industry. As with crown gall, there are no effective control measures for DBC. Consequently, ARS scientists are designing and developing robust detection methods for *Brennaria*.

Exotic prokaryotic pathogens of citrus, cereals, watermelon, grape, shade trees, potato tomato, grass seed, and rice*:* Foreign species of plant-pathogenic bacteria have become a major threat to U.S. agriculture because of the increase in air travel and international agricultural trade. The accidental or deliberate release of a regulated bacterial plant pathogen could result in severe economic damage. Researchers used an unpublished, newly developed pathogen-rating software program to determine the threat of naturally or deliberately released pathogens. The researchers then selected four high-threat foreign bacteria: *Rathayibacter* species, *Xylella fastidiosa*, *Xanthomonas campestris* pv. c*itri*, and *Xylophilus ampelinus*.

Molecular and Morphological Systematics of Plant-Pathogenic Fungi: Economic loss to agricultural and horticultural crops due to disease-causing fungi is estimated at $20 billion per year in the United States. In addition, fungi often limit U.S. agricultural exports, a significant factor in the U.S. balance of trade. Many of these pathogens have several morphologically similar relatives with no quarantine significance. Rapid and accurate methods for identification of quarantine significant fungi are critical to protect U.S. export markets. The $6 billion wheat export market was threatened by the presence of Karnal bunt and morphologically similar fungi, including a bunt fungus on ryegrass found throughout the southeastern United States. Other beneficiaries of similar research include the soybean, fruit, and tree nut industries.

Phytophthora spp. of strawberry, nut trees (walnuts/almonds), stone fruit and grape: This project focuses on determining the etiology of and improved management strategies for key diseases of small fruits and deciduous tree crops includes the development of improved diagnostic detection methods for pathogenic *Phytophthora spp.* affecting strawberry and deciduous fruit and nut crops. PCR primers are being used for specific amplification of mitochondrial DNA from *P. cactorum, P. citricola, and P. megasperma* extracted from soil and plant samples.

Alternaria diseases of fruit trees: Comprehensive knowledge of the biology of microorganisms associated with tree fruits, including pathogen taxonomy, disease etiology, and disease detection is lacking. Such knowledge is critical for development of science-based phytosanitary regulations and mitigative measures for imported and exported tree fruits. Mis-identification of fungal and bacterial pathogens of tree fruits can lead to the importation of exotic pathogens and diseases, and can hinder progress in development of export markets for U.S. commodities. To address these issues ARS scientists are studying pathogen biology of selected agents, including *Alternaria* spp. (causing several different fruit diseases) and are conducting morphotaxonomic and DNA

fingerprinting studies necessary to develop DNA-based methods for detecting and identifying exotic and domestic fruit pathogens in this genus.

Fungal diseases of cotton: Plant pathogens and nematodes cause annual yield losses of between 11 and 13%, accounting for over $800 million in losses to U.S. cotton growers per year. Despite the introduction of new elite varieties, cotton yields have been stagnant for over a decade. The causes of yield stagnation are varied and complex, but the emergence of new pathogens, the spread of recognized pathogens, and the tenacious resiliency of established pathogens are significant factors. Specific pathogens or diseases include the reniform nematode, race 4 of *Fusarium oxysporum* f. sp. *vasinfectum*, bronze wilt, South Carolina seed rot, and seedling diseases caused by *Rhizopus oryzae* and other pathogens. The multi-prong attack strategy required to combat these problems includes the development of resistant varieties, the identification of pathogenic organisms, the development of effective biocontrol agents, and the acquisition of a knowledge base encompassing both the mechanisms of pathogenicity and the factors affecting plant resistance.

Soybean rust: Soybean rust is an important disease causing significant yield losses in most soybean growing regions throughout the world, except for the United States. At least two species of *Phakopsora* cause soybean rust. Asian isolates of the soybean rust fungus *Phakopsora pachyrhizi* have been reported to be more virulent and aggressive than those of *P. meibomiae* from South and Central America and Puerto Rico. The Asian species of soybean rust has been estimated to result in yield losses of over 10% if it becomes established in the continental U.S. In November 2004, soybean rust was found in the United States for the first time, and since then has spread to 9 states.

S*oilborne fungal pathogens of alfalfa and dry beans:* Alfalfa is the fourth most important crop in the United States in terms of both cultivated acreage and production value. Approximately 25% of the alfalfa hay crop of the United States is lost annually to disease, principally the result of soilborne pathogens. Conventional chemical approaches to disease control are too expensive, ineffective, or deleterious towards non-target organisms and the environment. The most effective methods for minimizing losses from disease are to avoid infested fields and to grow alfalfa cultivars that are resistant to multiple diseases. Timely detection of pathogens in fields using microbiological methods is impeded by an unavailability of selective media for specific pathogens, or the long incubation periods required for unambiguous pathogen identification. PCR-based assays are needed to accurately and quickly detect soilborne pathogens of alfalfa in infected plants and infested field soil. Real-time PCR assays that quickly and reliably quantify pathogen biomass in infected tissues would facilitate the accurate selection of highly resistant plants.

Sudden oak death and the ornamentals industry: Sudden oak death (SOD) is caused by *Phytophthora ramorum*, a newly described species found in 1993 to attack ornamentals in Germany and the Netherlands. The same pathogen (as determined by morphology and isozyme analysis) has been known since the mid-1990's to be responsible for death of thousands of oaks in California. Sudden oak death has become of major concern to the

ornamentals industry and other agricultural industries because of state, federal, and international quarantines (Canada) affecting the movement of susceptible, high-value nursery crops such as rhododendrons and azaleas, as well as firewood and other plant-related products. The list of hosts of *P. ramorum* is now over 30 in number and growing, but little is known about the susceptibility of potential host species in the Eastern United States. Because several west coast nurseries unknowingly shipped thousands of infected plants throughout the United States in 2003 and 2004, major efforts are underway to prevent *P. ramorum* from becoming established in the East.

Molecular and Morphological Systematics of Plant-Pathogenic Nematodes: Plant-parasitic nematodes are microscopic soil worms that reduce the yield and quality of many crops, with annual agricultural losses of over $10 billion in the United States. Accurate and rapid identifications are urgently needed to determine the extent of nematode damage and to develop nematode control strategies. Because identification can be a difficult and time-consuming process, ARS scientists are improving the science of nematode identification through compiling guides or "keys" for identifying nematodes, updating lists of crops damaged by specific nematodes, maintaining nematode collections, determining new traits useful for identifying nematodes, and placing them in family trees in order to predict their agricultural effects. In addition, some beneficial nematodes from soil and insects are being similarly characterized. Identifications of deterious nematodes from plant materials introduced to or exported from the United States limit the international and interstate spread of nematodes and permit export of billions of dollars of agricultural products monitored by regulatory agencies. Although fruit and nut trees, ornamentals, potatoes, soybean, vegetables, and wheat are the major crops impacted by ARS nematode systematics activities, the scope of this project is truly multicommodity.

Soybean cyst nematode: Each year, the quantity and quality of soybean produced in the United States are adversely affected by a wide range of pathogens and pests, which cause a billion dollar crop loss annually. Disease-causing pathogens are being characterized in order to produce new soybean lines that are resistant to diseases. Investigations are being done to identify host genes that permit the vertical transmission of viruses and virus genes that reduce seed quality. These investigations will reduce the impact of diseases on soybean production by enhancing soybean germplasm with resistance to pathogens, by creating knowledge of the host-pathogen interactions that lead to disease, and by developing sustainable agricultural practices.

Exotic viruses of stone fruits and citrus: Exotic viruses of stone fruits and citrus and their potential insect vectors pose threats to our agricultural system through natural or intentional introductions. The protection of our agricultural production systems depends on the development of detection technologies, identification of causal agents and potential mechanisms of transmission, and determination of factors required for disease management, eradication, or prevention. Because viruses readily mutate and genetically recombine, understanding the dynamics of population change improves the probability of control, prevention, and eradication.

Viruses of citrus, vegetables, ornamentals and stone fruits: Multiple pathogens are responsible for production losses in the U.S. citrus, vegetable, ornamental and stone fruit industries. New and/or more severe plant diseases may indicate the existence of new plant pathogens or the appearance of more virulent strains of existing pathogens. In both cases, the synthesis of useful tools that diagnose and differentiate pathogens requires the integration of data on the biological, serological and genetic attributes of these pathogens. The successful management of existing disease problems can benefit from new diagnostic tools developed with data generated from the application of new genetic technologies to pathogen identification and differentiation not possible using previous research capabilities.

***Viruses of floral and woody ornamental plants*:** Many plant virus diseases cause significant losses in the production and quality of ornamental crops. These diseases are very difficult to control, and new diseases occur as different crops are introduced or grown in new areas. Many crops are susceptible to multiple viruses, each of which may cause serious economic losses, and infected plant material may not be acceptable for sale or export. Methods to reliably and rapidly detect and identify these viral pathogens are necessary for the production of virus-free or virus-indexed plants. One primary focus of ARS research is on those "new" currently uncharacterized or emerging viruses affecting key ornamental crops recently identified as significant to the floral and nursery industry. An understanding of the identity of new and emerging ornamental viruses, as well as their mechanisms of infection, transmission, and pathogenicity, is needed in order to develop better methods of disease control.

Exotic viruses of sweet potatoes: Federal regulations prohibit the entry of sweet potato germplasm from foreign sources for propagative purposes. Small quantities of germplasm are allowed for scientific uses, if the material undergoes quarantine and indexing for exotic pathogens (primarily viruses and phytoplasmas). ARS is responsible for conducting the quarantine and testing of sweet potato germplasm. Sweet potato virus disease (SPVD) is among the most serious diseases of sweet potato worldwide. Previously, it was thought to be caused by a synergistic interaction of two viruses, sweet potato feathery mottle virus (SPFMV, a potyvirus) and sweet potato chlorotic stunt virus (SPCSV, a crinivirus). However, the etiology of the disease is poorly understood and considerable variation has been reported in the symptoms and severity. In order to find effective control, elimination, or prevention methods for the disease, a better understanding of the disease etiology is needed.

***Grapevine rootstock stem lesion complex and young vine decline of grape*:** In grapevines, young vine decline (YVD) was reported in the early 1990's and was found to be associated with several fungal pathogens. However, ARS researchers have discovered a new virus strain and several new graft-transmissible agents (GTAs) that have significant impacts on vine health and productivity and are associated with YVD. Researchers are also discovering and characterizing new GTAs that are involved in another problem in grape production, grapevine rootstock stem lesion.

Viruses of strawberry, blueberry, blackberry and raspberry: The yield and quality of small fruit crops in the United States are adversely affected by viruses transmitted by soilborne and aerial vectors. Viruses have been implicated in decline symptoms in blackberry production fields in the southern and southeastern United States, black raspberry fields in the northwest and north central United States, and strawberry fields in California, Oregon, Washington and the Northeast, as well as in the newly described "fruit drop" and "no blossom" diseases in blueberry. In each of these crops, the available laboratory tests for viruses failed to detect any virus associated with the disease symptoms. Lack of adequate knowledge about the etiology and epidemiology of these diseases precludes development of environmentally sound, effective and economic disease control management strategies. There is a need to develop rapid assay procedures for the uncharacterized viruses in the small fruit crops so that the health status of plants can be determined in a timely manner.

***Rhizomania of sugarbeet and other viruses of sugarbeet, lettuce, tomato, cucurbits, and Strawberry*:** Several economically significant soilborne and insect-transmitted virus diseases affect the sugarbeet and vegetable industries of California, the rest of the United States, and the world. Previously unknown viruses continuously emerge and cause significant economic losses, while older viruses re-emerge to once again impact production. The emergence of new viruses has been exacerbated in recent years by the international movement of plant materials and their vectors, increased farming, farming in new areas, and global climatic changes. ARS research is directed at detection and identification of new viruses as they emerge in affected crops. Specific biological and molecular approaches are being used to elucidate the etiology and molecular evolution of sugarbeet, vegetable and interrelated crop and weed viruses.

Multidisciplinary: The Electron Microscopy Unit: A wide range of commodities are impacted by the research of the EMU at the Beltsville Agricultural Research Center (BARC), including soybean, potato, vegetables, grain crops, cacao, coffee, citrus, and weed species. The mission of the EMU is (i) the application of an array of electron microscopy techniques to the research needs of scientists at Beltsville and other locations and (ii) the development of new techniques and methodologies in electron microscopy for achieving previously unobtainable data. Standard scanning electron microscopic observation is carried out after solvent dehydration, critical point drying and heavy metal sputter coating. This procedure often yields specimen materials that are collapsed, detached from their host materials and cleansed of any soluble surface materials making accurate taxonomic description and host/pathogen relationships difficult to accurately observe. Consequently, the EMU is developing improved methods of specimen preparation and fixation. Although this work spans all of the projects studied at BARC, it does focus on plant pathogens and pests.

Discovery Area 1: Identification of Pathogens New to Science or Unknown in the United States

Fungi

Alternaria diseases of fruit trees

Accomplishment: At the request of APHIS-PPQ, from 2001-2003 an ARS scientist examined fruit from multiple shipments of Ya Li pears from China that were intercepted at a U.S. port-of-entry because of extensive decay caused by *Alternaria*. Isolations of *Alternaria* were made from the fruit, and pathogenicity testing was completed with representative Chinese strains and with other fruit-associated *Alternaria* species. After morphological and genetic characterization of the Chinese strains, the causal agent was determined not to be *A. gaisen* nor *A. alternata* (as claimed by the Chinese government) but instead was at least two undescribed species unknown in the United States. One of these was recently described as the new species *A. yaliinficiens*. This species is now known as one of the etiologic agents of the disease now referred to as chocolate spot of Ya Li pear.

Impact: This work has protected the U.S. pear grower from introduction of potentially damaging exotic fruit pathogens. The Chinese Ya Li pear import program has been opened and then suspended every year since 2001 because of repeated interception of the exotic *Alternaria* spp. On December 15, 2003, an ARS scientist discovered in a Wenatchee, WA retail outlet Chinese Ya Li pear fruit that had passed the import inspection procedure but possessed symptoms of chocolate spot disease. On December 19, 2003 APHIS issued a nationwide recall notice for all Ya Li pear fruit of Chinese origin. Within 24 hours of the notification to APHIS, the presence of diseased Ya Li pears was confirmed in 18 cities across the United States. APHIS smuggling interdiction teams were dispatched across the United States; the teams seized, destroyed, or allowed re-export of more than 3.25 million pounds of imported Chinese Ya Li pears in the United States. Diseased fruit was detected in 666 retail outlets. More than 60 sea containers of Ya Li fruit en route from China were refused entry into the United States, and this import program remains closed today, pending new information on the biology of the pathogens and development of effective mitigative measures.

Documentation:

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presumed asexual species in the filamentous Ascomycete genus *Alternaria*. Mycological Research. 2003. V. 107(2). P. 169-182.

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Fungal diseases of cotton

Accomplishment: ARS scientists conclusively established that two species of *Pantoea* are etiological agents that cause South Carolina Seed Rot. *Rhizopus oryzae* was identified as a virulent soil-borne pathogen inciting pre-emergence damping-off in susceptible cotton cultivars. A unique and highly virulent strain of *Fusarium oxysporum* f. sp. *vasinfectum* was discovered in cottonseed imported from Australia into California as a feed for dairy cows. This strain has devastated cotton production in areas of Australia where it occurs, resulting in 98% yield losses.

Impact: Identification of the causal agent of South Carolina Seed Rot is the first step required to develop strategies to control this disease. Identification of the highly virulent isolate of *Fusarium oxysporum* f. sp. *vasinfectum* from Australia resulted in the initiation of steps to more thoroughly disinfest cottonseed before it is allowed into the U.S.

Documentation:

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Nematodes

Molecular and Morphological Systematics of Plant-Pathogenic Nematodes

Accomplishment: Since 2000, ARS has provided taxonomic expertise for 2,724 nematode samples received from APHIS personnel (406 urgent samples and 682 non-urgent) and federal, state and foreign scientists for research, regulatory and control purposes. In response to a request from APHIS for species identification, ARS examined the ribosomal intergenic spacer (IGS) and mitochondrial sequences of a suspicious root-knot nematode from Florida and confirmed that it was *Meloidogyne mayaguensis*, a resistance-breaking tropical species not previously found in the continental United States. Descriptions of new species of lesion nematode, cyst nematode, needle nematode and two

root-knot nematodes incorporating morphological, molecular and host characterizations were made, thereby identifying the causal agents of newly discovered crop losses.

Impact: Identifications for APHIS personnel allowed port-of-entry personnel to prevent the spread of dangerous nematodes and also aiding the export of millions of dollars of U.S. agricultural products. Confirmation of the identity of *M. mayaguensis* resulted in increased sampling for this resistance-breaking nematode; multiple detections in Florida have resulted in changes in agronomic practice. Morphological plus molecular descriptions for new species led to control recommendations for new diseases and increased the reliability of species diagnoses by all nematode diagnosticians.

Additional Information: Collaboration with APHIS has been a major source of live material and identification requests behind these accomplishments. Several university researchers have also informally contributed materials and expertise. APHIS provides funding each year to partially defray the costs of providing identifications.

Documentation:

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Handoo, Z.A., Carta, L.K., Skantar, A.M. 2001. Morphological and molecular characterization of Pratylenchus arlingtoni n. sp., P. convallariae and P. fallax (Nemata: Pratylenchidae). Nematology. V. 3 p. 607-618.

Viruses

Viruses of citrus, vegetables, ornamentals and stone fruits

Accomplishment: A novel tobamovirus was isolated from symptomatic hibiscus in Florida nursery stock and landscape plantings, characterized and subsequently recognized as a new species, Hibiscus Latent Fort Pierce Virus (HLFPV). Detection strategies have been developed, tested and transferred.

Impact: HLFPV and a recently discovered tobamovirus from Singapore are the first well-characterized tobamoviruses known to infect malvaceous hosts, including cotton, okra and kenaf. This discovery has extended virological knowledge and necessitates a revision of the tobamovirus genus to accommodate the addition of a new subgroup.

Additional Information: Major collaborations have included University of Florida-Citrus Research and Education Center, Florida Department of Agriculture and Consumer Services Division of Plant Industry and APHIS-PPQ.

Documentation:

Kamenova, I., Adkins, S. 2004. Comparison of detection methods for a novel tobamovirus isolated from Florida hibiscus. Plant Disease. V. 88 p. 34-40.

Adkins, S., Rosskopf, E.N. 2002. Key West nightshade, a new experimental host for plant viruses. Plant Disease. V. 86 p. 1310-1314.

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Viruses of floral and woody ornamental plants

Accomplishment: Using serological and molecular technologies, ARS scientists determined the identity of and performed the initial characterization of several of new and emerging viruses of floral and woody ornamental plants, and produced reagents and tools for their detection and diagnosis. 1. A Pea mosaic strain of Bean yellow mosaic potyvirus was identified for the first time by serology, cloning and sequencing; this virus was found in a mixed infection with broad bean wilt fabavirus causing a mosaic disease in *Verbena*. 2. Two new potyviruses infecting the orchid *Spiranthes* were discovered and portions of their genomes were sequenced. 3. Two additional potyviruses, one associated with flower break symptoms in New Guinea *Impatiens* and the other associated with leaf mosaic in *Omphalodes*, were discovered and partially characterized, and their 3' terminal genomes were sequenced. 4. The complete nucleotide sequence of a Gladiolus isolate of Bean yellow mosaic virus (BYMV) was determined. 5. Strains of Alternanthera mosaic potexvirus (AltMV) infecting creeping phlox and trailing Portulaca were also identified, with one isolate from phlox completely cloned and sequenced and the 3' regions of two additional phlox and one Portulaca isolate sequenced. 6. A previously uncharacterized carlavirus from creeping phlox has also been completely cloned and sequenced, and characterization continues. 7. A tobamovirus isolate infecting *Petunia* has been partially cloned and sequenced, and shown to be distinct from other characterized isolates. 8. Polymerase chain reaction tests were developed for detection of AltMV, a newly identified phlox carlavirus, a presumptive carmovirus from *Angelonia*, and the Petunia tobamovirus. 9. Calla lily chlorotic spot virus and Calla lily latent virus were identified in diseased calla lily plants and were shown to be new, distinct tospovirus and potyvirus members, respectively; carnation mottle virus was identified for the first time in diseased calla lilies showing yellow mottling, light yellow spots and mosaic symptoms. 10. Amaryllis mosaic, Lycoris mild mosaic viruses and two yet to be named Lycoris potyvirus and Bacopa ilarvirus are being investigated 11. Other unknown or previously undescribed emerging viruses causing diseases in *Amorphophallus, Angelonia, Bacopa, Cyrtanthus, Lachenalia, Nandina, Ornithogalum, Phlox, Scaveola, Tricyrtis* and *Viola* are currently under investigation.

Impact: Other than natural or engineered resistance (which is rare), the prevention of disease through selection of pathogen-free or pathogen-indexed plants is the best method of controlling viral diseases. Identification of these new and emerging viruses of ornamentals and the subsequent development of diagnostic reagents for virus detection protocols will allow U.S. floriculture companies and other growers to test propagation stock in order to select healthy plants, resulting in increased productivity, quality, and customer satisfaction. Improved diagnostic reagents will also help protect domestic crops from existing and foreign pathogens. Coat protein and antisense RNA constructs were used to transform *Gladiolus* to investigate virus resistance.

Additional Information: Three Specific Cooperative Agreements funded through the Floral and Nursery Research Initiative support research on other viruses of interest to the floral industry. In addition, under an SCA with the University of California-Riverside, a tymovirus affecting *Diascia, Nemesia*, and *Verbena* has been partially characterized, an antiserum was produced for detection, and experiments were conducted to identify the optimum tissues to sample for reliable detection. The host range of the virus was examined, and the double-stranded RNA profile of the virus determined, allowing specific detection of the tymovirus in mixed infections. A second virus is also being characterized. Another SCA, with Ohio State University, has examined the distribution of a tobamovirus in *Petunia*, and the environmental conditions that lead to greatest reliability of detection by ELISA. The third SCA, with Oregon State University, has focused on the detection of a potyvirus in *Verbena*, and the ability to detect the virus during tissue culture propagation. The virus was identified as a pea mosaic virus isolate of Bean yellow mosaic virus, and it was found to be reliably detected by an ELISA using a cross-reactive monoclonal antibody previously developed by ARS. Work on characterization of a carmovirus in *Angelonia* is being carried out in collaboration with ARO, Bet Dagan, Israel.

Documentation:

Hammond, J. and Jordan, R.L. 2001. Potyviruses. in: Encyclopedia of Plant Pathology. John Wiley & Sons, New York. pp. 792-800.

Hammond, J., Hammond, R. 2003. The complete nucleotide sequence of Bean yellow mosaic virus isolate BYMV-GDD and comparison to other potyviruses. Archives of Virology. v.148 p. 2461-2470.

Nemchinov, L.G., Hammond, J., Jordan, R.L., Hammond, R. 2004. The complete nucleotide sequence, genome organization, and specific detection of Beet mosaic virus. Archives of Virology. v.149 p. 1201-1204.

Kamo, K.K., Gera, A., Cohen, J. Hammond, J., Blowers, A., Smith, F., van Eck, J. 2005. Transgenic Gladiolus plants transformed with either the bean yellow mosaic virus coat protein or antisense RNA. Plant Cell Reports v.23:654-663.

Hsu, H. T., Ueng, P. P., Chu, F. H., Ye, Z., and Yeh, S. D., 2000. Serological and molecular characterization of a high temperature-recovered virus belonging to tospovirus serogroup IV. J. Gen. Plant Pathol. 66:167-175.

Chen, C.C., Chen, Y.K., and Hsu, H.T., 2000. Characterization of a virus infecting lisianthus. Plant Disease 84:506-509.

Hsu, H. T., Barzuna, L., Hsu, Y. H., Bliss, W., and Perry, K. L., 2000. Identification and subgrouping of Cucumber mosaic virus with mouse monoclonal antibodies. Phytopathology 90:615-620.

Gera, A., Hsu, H.T., Cohen, J., Watad, A., Beckelman, and Hsu, Y.H., 2000. Effect of cucumber mosaic virus monoclonal antibodies on virus infectivity and transmission by Myzus persicae J. Plant Pathol. 82:119-124

Aebig, J.A., Kamo, K., and Hsu, H.T., 2005. Biolistic inoculation of gladiolus with cucumber mosaic cucumovirus. Journal of Virological Methods. J. Virol. Methods. 123:89-94.

Exotic viruses of sweet potatoes, small fruits (gooseberries and currants) and stone fruits

Accomplishment: ARS scientists demonstrated that Sweet potato virus disease (SPVD) could be caused by a synergistic interaction of Sweet potato chlorotic stunt virus (SPCSV) with any one of several different potyviruses that infect sweet potatoes, not just sweet potato feathery mottle virus (SPFMV). Furthermore, the research showed that the severity of the disease symptoms varied with the individual potyvirus component of the mixed infection. This was the first report of potyviruses, other than strains of SPFMV, interacting with SPCSV to cause SPVD.

Impact: PCSV is not known to occur in commercial productions of sweet potato in the United States. However, three potyviruses are now known to be present. All three of the viruses were shown to interact with SPCSV to produce SPVD. One of these viruses, Sweet potato vein mosaic virus, was shown to cause an even more severe form of SPVD than that produced by the classical SPFMV/SPCSV mixed infection. Therefore, if SPCSV were introduced into the United States in infected germplasm, the economic impact to sweet potato production could be serious. This new knowledge will be valuable to breeders who are developing new varieties with resistance to SPVD.

Documentation:

Lotrakul, P., Valverde, R., Clark, C., Hurtt, S., Hoy, M. 2002. Sweet potato leaf curl virus and related geminiviruses in sweetpotato. Acta Horticulturae 583: 135-141.

Grapevine rootstock stem lesion complex and young vine decline of grape

Accomplishment: Graft-inoculations onto test plants of Cabernet Sauvignon scions on hybrid grape rootstocks, those resistant to Phylloxera strain B and used commercially in California vineyards, served as a differential host range to identify a lethal virus strain and several lethal GTAs as part of the Grapevine rootstock stem lesion complex. The lethal virus strain in Redglobe (RG) table grape was cloned and sequenced and identified as a variant of Grapevine leaf roll associated virus 2 (GLRaV-2); it was designated as GLRaV-2RG and killed graft-inoculated test plants on rootstocks 3309C, 5BB, 5C, 1103P and 1616C. An RT-PCR assay was developed for GLRaV-2RG and was tested on diseased wine grape samples; ca. 20% of them tested positive. Results of three test trials identified six additional grape sources with lethal GTAs. Their molecular characterizations are in progress.

Impact: A rapid assay for only GLRaV-2RG has been developed and three new biological indicators have been identified to detect all lethal GTAs discovered to date. They are 3309C, 5BB and 5C. These additions will be proposed for inclusion in the California grapevine clean stock program.

Additional Information: The discovery of new GTAs in grape was achieved through collaboration with Dr. Adib Rowhani (UC Davis) and various postdoctoral associates funded through the Improvement Advisory Board, the California Competitive Grant Program for Research in Viticulture and Enology, and a CPGR Specific Cooperative Agreement (SCA) with PI Adib Rowhani. Rootstock trials are conducted at the Armstrong Tract in collaboration with the Department of Plant Pathology, UC Davis.

Documentation:

Rowhani, A., Zhang, Y.-P., Golino, D.A., and Uyemoto, J.K. 2000. Isolation and partial characterization of two new viruses from grapevine. Proceedings 13th Meeting International Council for Virus and Virus-like Diseases of Grapevines. Adelaide, Australia. pp. 83-84.

Uyemoto, J. K., Rowhani, A., Luvisi, D., and Krag, C.R. 2001. Discovery of a new closterovirus in Redglobe grape causing decline of grafted plants. California Agriculture 55(4): 28-31.

Uyemoto, J.K. and Rowhani, A. 2003. Discovery of different grapevine sources with graft- transmissible agents causing union-incompatibility on sensitive rootstocks. Proceedings 14th International Council for Virus and Virus-like Diseases of Grapevines. Locorotondo, Italy. pp. 139-140.

Rowhani, A., Uyemoto, J.K., Golino, D.A., and Martelli, G.P. 2005. Pathogen testing and certification of Vitis and Prunus species. Annual Review of Phytopathology 43: (accepted).

Westphal, A., Browne, G.T., and Schneider, S. 2002. Evidence for biological nature of the grape replant problem in California. Plant and Soil 242:197-203.

Discovery Area 2: Identification or Genetic Characterization of Known Pathogens or New Strains of Known Pathogens

Prokaryotic Plant Pathogens

Floral and woody ornamental plant prokaryotes

Accomplishment: ARS scientists discovered the association of *Xylella fastidiosa* with disorders of three hosts and produced the first report of bacterial leaf scorch in a bonsai tree. ARS scientists also demonstrated the association of *X. fastidiosa* with a leaf-scorch disorder in Japanese beech bonsai and in black oak in the United States, as well as the causal role of *X. fastidiosa* in oleander leaf scorch and the presence of the disease in various locations in Texas. In order to determine how *X. fastidiosa* strains from alternative hosts are related genetically to other hosts, ARS scientists first isolated *X. fastidiosa* from its alternative hosts (porcelain berry and wild grape) and then determined their genetic relationships with each other and with strains from grape, peach, plum, oak, mulberry, maple and oleander. These alternative host strains of *X. fastidiosa* were discovered to be more closely related to the oak strain than the grape strain.

ARS scientists also determined that symptomless geranium plants infected with *Ralstonia solanacearum* race 3 biovar 2 (R3bv2) release easily detectable numbers of bacteria from their roots; this discovery is being developed into a rapid field screening method.

Impact: The finding that *X. fastidiosa* is associated with high-valued bonsai is significant because it is an important step leading to the control of the disorder and preservation of the horticultural masterpiece. The discovery is also of great value for regulatory officials when bonsai plants are moved between countries, particularly from the United States to Asian countries where *X. fastidiosa* has not been found. The findings that *X. fastidiosa* is associated with black oak and causes oleander leaf scorch in Texas expand the host range of the bacterium in economically important landscape tree species, and extend the geographic range of this important bacterial disease, respectively. The phylogenetic study suggests that alternative hosts may play an important role in the spread of *X. fastidiosa* to economically important hosts such as oak by serving as a reservoir of inoculum. Removal of these alternative host plants may be important for disease control.

The examination of bacterial wilt-host relationships with *Ralstonia solanacearum* Race 1 as a model system has led to the screening of bactericides for their ability to protect geranium plants from infection. The beneficiaries of the *Ralstonia* research are the geranium production industry, the hundreds of U.S. greenhouse ornamental growers that grow and sell geraniums, and APHIS-PPQ, the regulatory agency responsible for developing policies that exclude and eradicate this Select Agent. Even though strict sanitation procedures have been implemented in geranium production facilities, a low

cost effective protectant would greatly benefit the $300 million/year geranium industry and will help safeguard the $1.2 billion/year U.S. potato industry.

Additional Information: Two Specific Cooperative Agreements (SCA) funded through the Floral and Nursery Research Initiative support research on *Ralstonia solanacearum.* One SCA with the University of Wisconsin-Madison and addresses research on the release of bacteria from symptomless plants; the other is with the University of Florida and examines bacterial wilt-host relationships.

Documentation:

Huang, Q., Li, W. and Hartung, J. S. 2003. Association of *Xylella fastidiosa* with leaf scorch in Japanese beech bonsai. Canadian Journal of Plant Pathology 25: 401-405.

Huang, Q. and Sherald, J. L. 2004. Isolation and phylogenetic analysis of *Xylella fastidiosa* from its invasive alternative host, porcelain berry. Current Microbiology 48: 73-76.

Huang, Q. 2004. First report of *Xylella fastidiosa* associated with leaf scorch in black oak in Washington, D. C. Plant Disease 88: 224.

Huang, Q., Brlansky, R. H., Barnes, L., Li, W. and Hartung, J. S. 2004. First report of oleander leaf scorch caused by *Xylella fastidiosa* in Texas. Plant Disease 88: 1049.

Fungi

Phytophthora spp. of strawberry, nut trees (walnuts/almonds), stone fruit and grape

Accomplishment: ARS scientists have characterized genetic (AFLP) and pathogenic diversity of *P. cactorum* and *P. citricola*, two pathogens that cause widespread damage on almond, strawberry, and many other California crops.

Impact: The pathogenic and genetic characterizations of populations of *Phytophthora cactorum* and *P. citricola* from diverse hosts will provide a clear picture of the population structure of these important pathogens. This is an essential component in an effective cultivar and rootstock breeding programs for *Phytophthora* resistance.

Documentation:

Browne, G.T., Viveros, M.A. 2005. Effects of phosphonate and mefenoxam treatments on development of perennial cankers caused by two *Phytophthora* spp. on almond. Plant Disease 89:241-249.

Browne, G.T., Wilcox, W.F., Latore. 2005. Phytophthora crown and root rot. In: Compendium of Grape Diseases. The American Phytopathological Society, St. Paul.

Nematodes

Soybean cyst nematode

Accomplishment: Soybean cyst nematode is able to overcome all known sources of resistance. A genetic linkage map was developed for soybean cyst nematode and markers for virulence to some sources of resistance were located on the map.

Impact: The genetic linkage map will provide for map-based cloning of virulence genes of soybean cyst nematode and will provide the framework for whole genome sequencing. This genetic linkage map is the second one developed for a plant-parasitic nematode and the first developed for SCN. This groundbreaking work is the most important research done thus far to understand the genetics of SCN, especially as it relates to parasitism and ability to defeat plant resistance.

Additional Information: Collaborative efforts with colleagues from the University of Illinois and outside funding provided personnel and resources to produce the first genetic linkage map of soybean cyst nematode and to place markers for virulence on the linkage map.

Documentation:

Atibalentja, N., Bekal, S., Domier, L. L., Niblack, T. L., Noel, and Lambert, K. N. A genetic linkage map of the soybean cyst nematode, *Heterodera glycines*. Molecular Genetics and Genomics. (Accepted 12/29/2004).

Viruses

Rhizomania of sugarbeet and other viruses of sugarbeet, lettuce, tomato, cucurbits, and strawberry

Accomplishment: Beet necrotic yellow vein virus (BNYVV) is the causal agent of rhizomania disease of sugar beet. In 2002-2004 in the Imperial Valley of California, resistant sugar beet cultivars containing the *Rz1* allele were severely affected by rhizomania and contained elevated virus levels. Distinct BNYVV isolates from Imperial Valley (IV-BNYVV) were identified from infected sugar beet roots by single local lesion isolation. Because these isolates do not contain RNA-5 as determined by RT-PCR and the banding patterns of single-strand conformation polymorphism analyses, we concluded that the resistance-breaking BNYVV isolates had likely evolved from the original existing A-type. The pathogenicity of IV-BNYVV isolates was studied, and PCR products from coat protein (RNA-2) and P-25 protein (encoded by BNYVV-RNA-3, involved in symptom expression) of IV-BNYVV isolates were sequenced. Sequence alignments from both coat protein and P-25 protein revealed only minor amino acid changes compared to the existing A-type of California BNYVV isolates.

Impact: This research demonstrated that minor genetic changes may have allowed the A-pathotype to overcome *Rz1*-mediated resistance. This knowledge is critical to development of new strategies for control of rhizomania throughout the world, through both traditional breeding and biotechnology. Furthermore, this research has provided important knowledge that has implications for prolonging the useful life of *Rz*- resistant germplasm in areas where resistance has not yet been overcome by virus evolution. Such rapid (only a few sugarbeet crops after beginning use of resistant germplasm) evolution of resistance-breaking pathotypes indicates it may be necessary to moderate the use of resistant germplasm in order to reduce the rate of emergence of resistance breaking pathotypes in other areas.

Documentation:

Wisler, G.C, Lewellen, R.T., Sears, J.L., Wasson, J., Liu, H.-Y. and Wintermantel, W.M. 2003. Interactions between *Beet necrotic yellow vein virus* and *Beet soilborne mosaic virus* in sugar beet. *Plant Disease* 87: 1170-1175.

Liu, H. Y., Sears, J. L., and Morrison, R. H. 2003. Isolation and characterization of a carom-like virus from *Calibrachoa* plant. Plant Dis. 87:167-171.

Wintermantel, W.M. 2005. Co-infection of *Beet mosaic virus* with beet yellowing viruses leads to increased symptom expression on sugarbeet. *Plant Disease* 89: 325-331.

Liu, H. Y., Sears, J. L., and Lewellen, R. T. 2005. Occurrence of resistance-breaking *Beet necrotic yellow vein virus* of sugar beet. *Plant Disease*. (in press/accepted for publication December, 2004).

Viruses of citrus, vegetables, ornamentals and stone fruits

Accomplishment: Considerable progress was made on two important insect-transmitted pathogens of citrus. The brown citrus aphid (BCA) is the most efficient vector of severe stem pitting isolates of citrus tristeza virus (CTV). ARS has separated several mild and severe CTV sub-isolates from mild field isolates of CTV. When sweet orange and Mexican limes were re-inoculated with known isolates, new and apparently recombinant forms were subsequently re-isolated. There are indications that the BCA may be selectively transmitting one strain versus others and/or the aphid serves as a bottleneck in separating virus population components. In addition, new genotypes of CTV were discovered.

Impact: This knowledge increases our understanding of the genetic diversity of CTV, provides data for improved control of CTV-caused diseases, will lead to better detection technologies, and will result in better screening of citrus germplasm.

Documentation:

Brlansky, R.H., Damsteegt, V.D., Howd, D.S., and Roy, A. 2003. Molecular analyses of Citrus tristeza virus subisolates separated by aphid transmission. Plant Disease 87:397-401**.**

Discovery Area 3: Development of New Methods for Identifying or Classifying Pathogens

Prokaryotic Plant Pathogens

Structural and functional genomics of potato, strawberry, grapevine, jujube, walnut, bean, carrot, peach, cherry, pear, and almond pathogens: Ultrasensitive PCR assays

Accomplishment: Highly sensitive means for detection of phytoplasmas and better understanding of phytoplasma ecology were needed for disease management, quarantine, and curbing disease spread. ARS scientists developed novel ultrasensitive nested-PCR assays for universal (all phytoplasmas), broad (specific group) and specific (specific subgroup) detection of phytoplasmas. New insights of phytoplasma ecology and biodiversity were achieved through use of these new molecular tools. Ultrasensitive PCR using universal primers permitted for the first time the detection of a broad array of phytoplasmas associated with plants and insect vectors. In mixed infections, specific nested-PCR allowed the detection of secondary phytoplasmas (often present in unusually

low concentrations) associated with many economically important woody plant species and some herbaceous plants; they were essentially undetectable by PCR assays with universal primers. This ultrasensitive method allowed for the first time the detection of multiple phytoplasmas in a single plant, thereby unraveling the complexity of phytoplasma diversity and ecology. Thus far, these molecular tools have been used to identify more than 20 new diseases and several new phytoplasma strains.

Impact: The development of "universal" primer pairs and nested-PCR assays permitted the broadest and most sensitive detection of phytoplasmas and made it possible to develop the first comprehensive classification of phytoplasmas based on 16S rDNA sequence analysis. These tools enabled researchers to detect and identify phytoplasmas that previously could not be detected. The nested PCR technologies for phytoplasma detection have been adopted by a diagnostic company (Agdia, Inc.) and by APHIS, as well as by research groups and diagnostic laboratories worldwide. This accomplishment has expanded the current knowledge of and changed the concepts of phytoplasma diversity and ecology.

Additional Information: A Trust Fund granted by AGDIA Inc. facilitated the design of PCR primers for the detection of phytoplasmas.

Documentation:

Abou-Jawdah, Y., Karakashian, A., Sobh, H., Martini, M., and Lee, I.-M. 2002. An epidemic of almond witches'-broom in Lebanon: Classification and phylogenetic relationships of the associated phytoplasma. Plant Dis. 86:477-487.

Lee, I.-M., Martini, M., Bottner, K. D., Dane, R. A., Black, M., and Troxclair, N. 2003. Ecological implications from a molecular analysis of phytoplasmas involved in an aster yellows epidemic in various crops in Texas. Phytopathology 93:1368-1377.

Lee, I.-M., Bottner, K. D., Munyaneza, J. E., Secor, G.A., and Gudmestad, N. C. 2004. Clover proliferation group (16SrVI), subgroup A (16SrVI-A) phytoplasma is probable causal agent of potato purple top disease in Washington and Oregon. Plant Dis. 88:429.

Liu, Q., Wu, T., Davis, R.E., and Zhao, Y. 2004 First report of witches'-broom disease of *Broussonetia papyrifera* and its association with a phytoplasma of aster yellows group (16SrI). Plant Dis. 88:770.

Montano, H.G., Davis, R.E., Dally, E.L., Pimentel, J.P., and Brioso, P.S.T. 2000. Identification and phylogenetic analysis of new phytoplasma from diseased chayote in Brazil. Plant Dis. 84:429-436.

Structural and functional genomics of potato, strawberry, grapevine, jujube, walnut, bean, carrot, peach, cherry, pear, and almond pathogens: Molecular systematics of phytoplasmas

Accomplishment: To date, phytoplasmas cannot be cultured in cell-free media. The traditional classification and taxonomy for walled bacteria based on biochemical properties and DNA-DNA homology is not applicable for phytoplasmas. Lack of rapid means for identification and classification of a broad array of phytoplasmas has hindered studies on the ecology of phytoplasmas and on the epidemiology of phytoplasmal diseases. ARS has evaluated the usefulness of conserved genes including 16S rRNA, ribosomal protein (rp), and secY, for classification of phytoplasmas. Broad-spectrum primers were designed for amplification of these genes from phytoplasmas. We devised and constructed a comprehensive classification scheme for a broad array of phytoplasmas (consisting of 15 phytoplasma groups and more than 50 subgroups) based on RFLP analysis of PCR-amplified 16S rDNA, rp, and secY gene sequences. This new scheme represents the most comprehensive classification system available that allows for the identification of numerous phytoplasmas worldwide. Three '*Candidatus* Phytoplasma species' have been proposed.

Impact: This accomplishment provided a rapid approach for identification of phytoplasmas that was adopted by scientists worldwide and a system recognized internationally peers as a major breakthrough for classification of phytoplasmas. As a result, the phytoplasma research field has dramatically expanded. The system is currently used by the National Center for Biotechnology Information (NBCI)/GeneBank for classification of phytoplasmas. This accomplishment has generated many collaborative research projects both nationally and internationally. The taxonomic proposal was a key breakthrough that launched phytoplasma taxonomic speciation and was adopted by the International Committee of Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes. Thus far, genus *Phytoplasma* has been officially proposed, and more than 20 *Candidatus* phytoplasma species have been published. The much-needed genomics information is also being used by California Department of Agriculture, in collaboration with ARS, to develop real-time PCR protocols for early detection of corn stunt epidemics; in California; an outbreak of the disease in 2002 caused damage exceeding $5 million in Kings County alone. On the practical aspect, these new discoveries have improved strain and species differentiation and classification, and will lead to development of new tools for pathogen detection and identification.

Documentation:

Montano, H. G., Davis, R.E., Dally, E.L., Hogenhout, S., Pimentel, J.P., and Brioso, P.S.T. 2001. '*Candidatus* Phytoplasma brasiliense', a new phytoplasma taxon associated with hibiscus witches'-broom disease. Int. J. Syst. Evol. Microbiol. 51:1109-1118.

Lee, I.-M., Gundersen-Rindal, D. E., Davis, R. E., Bottner, K. D., Marcone, C., and Seemüller, E. 2004. '*Candidatus* Phytoplasma asteris', a new phytoplasma taxon associated with aster yellows and related diseases. Int. J. Syst. Evol. Microbiol. 54:1037-1048.

Lee, I.-M., Martini, M., Marcone, C., and Zhu, S. F. 2004. Classification of phytoplasma strains in the elm yellows group (16SrV) and proposition of '*Candidatus* Phytoplasma

ulmi' for the phytoplasma associated with elm yellows. Int. J. Syst. Evol. Microbiol. 54:337-347.

Jomantiene, R., Davis, R.E., Valiunas, D., Alminaite, A., and Staniulis, J. 2002. New group 16SrIII phytoplasma lineages in Lithuania exhibit rRNA interoperon sequence heterogeneity. Europ. J. Plant Pathol. 108:507-517.

Lee, I.-M., Davis, R.E., and Gundersen-Rindal, D.E. 2000. Phytoplasma: Phytopathogenic Mollicutes. Annu. Rev. Microbiol. 54:221-255.

Exotic prokaryotic pathogens of citrus, cereals, watermelon, grape, shade trees, potato, tomato, grass seed, and rice

Accomplishment: Real-time PCR assays for rapid, routine detection of several high-risk bacterial pathogens were developed. A real-time BIO-PCR protocol was developed for detection of the APHIS Select Agent *Ralstonia solanacearum* race 3, biovar 2 in asymptomatic potato tubers within 48 hours. A direct (i.e., without DNA extraction) real-time PCR assay was developed for rapid, on-site diagnosis of Pierce's disease in grape, leaf scorch in shade trees, and citrus variegated chlorosis (CVC) in citrus. The method employs dry beads containing all PCR ingredients, including Taq polymerase, primers and probe for use with a portable PCR unit. The citrus canker bacterium *Xanthomonas campestris* pv. *citri* was detected in confiscated leaf and fruit samples on-site at San Francisco (SFO) and Los Angeles International (LAX) airports, by use of a portable PCR unit and newly developed real-time PCR primers and probe. A real-time BIO-PCR assay was developed for detecting *Acidovorax avenae* subsp. *avenae* in rice seeds. Over 50 cultures of Russian bacteria were received, including *Pseudomonas atrofaciens, X. oryzae* pv. *oryzae* and *R. solanacearum* race 3, biovar 2 from present and past projects.

Impact: The real-time BIO-PCR assay for *R. solanacearum* bv2 is being utilized in Russia, Turkey, and parts of Europe for routine assays of potato tubers. Our real-time PCR assay containing dry beads with all PCR ingredients is available commercially through Cepheid, Sunnyvale, CA for rapid, 1-2 hour on-site detection of the devastating exotic CVC bacterium in citrus, Pierce's disease in grape, and leaf scorch disease in many shade trees. When these diseases are diagnosed before they become established, control is achievable. Use of the real-time PCR assay for *X. citri* has demonstrated that real-time PCR can be used on-site at U.S. Ports in a routine manner and has established that citrus canker is entering California daily through SFO and LAX airports. This shows that molecular-based detection technologies can be used by port inspectors for rapid, on-site identification of diseased plants brought in by airplane passengers and in commercial shipping containers. By obtaining several cultures from past and present programs in Russia we have made available cultures to be fingerprinted and added to a database of high-risk plant pathogens. Making these rapid, specific PCR assays for high-risk pathogens available has significantly improved the security of U.S. agriculture. Moreover, the BIO-PCR method was patented (U.S. patent no. 6,410,223 issued on 25 June 2002) and is being used in many routine assays in several diagnostic and research fields, including food safety and environmental studies.

Additional Information: Collaborative research with CDFA and APHIS, SFO facilitated the ability to obtain current and archived citrus canker leaf and fruit samples at LAX and SFO. A scientist from Turkey with support from NATO spent a one-year sabbatical with ARS to assist in developing the real-time PCR assay for detecting *R. solanacearum* biovar 2 in potato. Cepheid, Sunnyvale, California collaborated in developing the rapid, on-site PCR assay for Pierce's disease and CVC by providing dry beads for real-time PCR free of charge. A Russian Scientist provided the cultures from Russia; this work in Russia would not have been possible without support from an ISTC grant; ISTC also supported a Russian scientist to spend six months at Ft. Detrick characterizing bacteria.

Documentation:

Schaad, N.W., Frederick, R.D., Shaw, J., Schneider, W.L., Hickson, R., Petrillo, M.D., and Luster, D.G. 2003. Advances in Molecular-Based Diagnostics in Meeting Crop Biosecurity and Phytosanitary Issues. Ann. Rev. Phytopathol. 41:305-324.

Ozakman, M. and Schaad, N.W. 2003. A real-time BIO-PCR assay for detection of *Ralstonia solanacearum* race 3, biovar 2, in asymptomatic potato tubers. Can. J. Plant Pathol. 25:232-239.

Schaad, N. W., Postnikova, E., Lacy, G., Fatmi, M., and Chung-Jan, C. 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. Syst. Appl. Micro. 27:290-300.

Song, W. Y., Kim, H. M., Hwang, C. Y., and Schaad, N. W. 2004. Detection of *Acidovorax avenae* ssp. *avenae* in rice seed using BIO-PCR. J. Phytopathol. 152: 667-676.

Mateeva, I.E.V., Pekhtereva, E. SH., Polityko, V.A., Ignatov, A.N., Nikolaeva, E.V., and Schaad, N.W. 2003. Distribution and virulence of *Pseudomonas syringae* pv. *atrofaciens*, causal agent of basal glume rot, in Russia. pp. 97-105. In: *Pseudomonas syringae* and related pathogens, N.S. Lacobellis, et al., (eds). Kluwer Academic Publishers, Dordrecht, The Netherlands (Book).

Crown gall and deep bark canker (DBC) of nut trees (walnuts/almonds)

Accomplishment: A real-time PCR detection and quantification system for *A. tumefaciens* residing in soil has been fully developed. PCR-based detection technologies for the bacterium *Brennaria rubrifaciens*, the causative agent of deep bark canker of walnut, have also been developed. Impact: Both of these new detection systems will aid in the development of sustainable control strategies for crown gall disease of walnut and almonds and deep bark canker of walnut. In addition, these detection tools are greatly aiding our efforts to examine the ecology of both *Agrobacterium* and *Brennaria* under

orchard conditions and will aid in walnut breeding programs designed to identify disease resistant selections.

Additional Information: Collaborators in the study of the ecology, biology and control of *Agrobacterium tumefaciens* include members of the faculty of the University of California-Davis and numerous growers and nursery operators. Research support has been obtained from the Almond Board of California, the Walnut Marketing Board, and the Improvement Advisory Board (IAB) of California.

Documentation:

Robertson, A. E., Wechter, W. P., Denny, T. P., Fortnum, B. A., Kluepfel, D. A. 2004. Relationship between Avirulence Gene (*avr*A) Diversity in *Ralstonia solanacearum* and Bacterial Wilt Incidence. Molec. Plant-Microbe Interactions 17: 1376-1384.

Fungi

Molecular and Morphological Systematics of Plant-Pathogenic Fungi

Accomplishment: Morphological and molecular characters were used to distinguish the ryegrass bunt fungus, *Tilletia walkeri*, from the Karnal bunt fungus, *Tilletia indica*. Five key morphological characters were discovered for the identification of Karnal bunt, and a rapid PCR-based test that can be done in any lab with a PCR machine was developed to distinguish the ryegrass bunt from Karnal bunt. Online morphological identification resources have also been made available for species of *Tilletia* in the United States.

Impact: Methods to accurately identify quarantine significant plant pathogens reduce unnecessary delays and expenses for U.S. grain exporters and allow USDA APHIS to issue accurate phytosanitary certificates. This work has contributed to the accurate identification of species of *Tilletia* found by APHIS surveys and APHIS PPQ identifiers. Because Karnal bunt can now be accurately identified and its geographic distribution is known, U.S. wheat can be exported worldwide without unnecessary delays or risk of rejection by the importing country. This work also helps protect areas where Karnal bunt is not known to occur by preventing movement of infected grain. Improved methods of identification of both bunt fungi and canker-causing fungi are the long-term objectives for this project.

Additional Information: Collaboration with Washington State University (WSU) contributed to this accomplishment. WSU provided specimens and expertise in the systematics of wheat bunt fungi. APHIS scientists also facilitated this work by providing specimens and information. Karnal bunt data has been incorporated into the Web-based database of the U.S. National Fungus Collections; the database incorporates data from 700,000 specimens, accurate scientific names and plant hosts and distribution for fungi in the United States, and over 500,000 reports of plant-associated fungi throughout the world. These data support plant quarantine officials in preventing the entry of invasive fungi estimated at causing $12-15 billion damage annually to crop plants and forests.

Documentation:

Crous, P.W. Van Jaarsveld, A.B., Castlebury, L.A., Carris, L.M., Frederick, R.D., Pretorius, Z.A. 2001. Karnal bunt of wheat newly reported from the African continent. Plant Disease V. 85 p. 561.

Levy, L., Castlebury, L.A., Carris, L.M. Meyer, R. and Pimentel, G. 2001. ITS sequence based phylogeny and PCR-RFLP differentiation of *Tilletia walkeri* and *Tilletia indica*. Phytopathology V. 91 p. 935-940.

Farr, D.F., Castlebury, L.A. and Rossman, A.Y. 2002. Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of Phomopsis from blueberry and cranberry in the eastern United States. Mycologia V. 94 p. 494-504.

Castlebury, L.A., Rossman, A.Y. Jaklitsch, W., Vasilyeva, L.N. 2002. A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences. Mycologia V. 94 p. 1017-1031.

Rossman, A.Y., Aime, M.C. Farr, D.F.. Castlebury, L.A., Peterson, K.R., Leahy, R. 2004. The coelomycetous genera *Chaetomella* and *Pilidium* represent a newly discovered lineage of inoperculate discomycetes. Mycological Progress V. 3 p. 275-290.

Soybean rust

Accomplishment: Research and technology transfer activities enhanced preparedness for the introduction of soybean rust into the United States. The Internal Transcribed Spacer (ITS) region was cloned, sequenced and analyzed from the two *Phakopsora* species that cause soybean rust. Approximately 80% nucleotide sequence similarity was observed. Utilizing differences within the ITS region, a PCR diagnostic assay was developed that can identify and differentiate between the two *Phakopsora* species that cause soybean rust. ARS also conducted several training workshops on soybean rust identification for plant disease diagnosticians from the five Regional Plant Disease Diagnostic Centers, as well as for members of the NC-504 committee on soybean rust.

Impact: Since its initial introduction in November 2004, the ARS PCR assay was used to confirm the presence of the Asian strain of soybean rust in nine continental U.S. states. Previously, the assay had been used to determine the presence of soybean rust in South Africa, Brazil and Paraguay. One of the participants in the ARS soybean rust training workshops was the first person to identify soybean rust in the continental United States. He attributes his ability to identify soybean rust as a direct result of the ARS training. The 800 lines exhibiting some resistance to soybean rust have been released to universities and public breeders to incorporate into their breeding programs. As a result of the success of the molecular diagnostic assay, its developers have been inundated with requests for soybean rust DNA. Several Material Transfer Agreements have been drafted with U. S. and foreign collaborators who wish to conduct PCR assays for soybean rust. These include Purdue University, Kansas State University, University of Wisconsin, Cornell University, University of Florida, Mississippi State University, Midwest Research Institute, Pioneer Hybrid, Oregon State University, and the Maryland Department of Agriculture.

Additional Information: The United Soybean Board contributed funding in several areas of soybean rust research. The North Central Soybean Research Program (NCSRP) has provided funding for antibody work with soybean rust.

Documentation:

Frederick, R. D., C. L. Snyder, G. L. Peterson, and M. R. Bonde. 2002. Polymerase chain reaction assays for the detection and discrimination of the soybean rust pathogens *Phakopsora pachyrhizi* and *P. meibomiae*. Phytopathology 92:217-227.

Pretorious, Z. A., Kloppers, F. J., and Frederick, R. D. 2001. First report of soybean rust in South Africa. Plant Dis. 85:1288.

Sudden oak death

Accomplishment: ARS scientists developed a PCR-diagnostic assay based on mitochondrial gene sequences for detecting *Phytophthora ramorum* with a high level of sensitivity and specificity.

Impact: Under a Material Transfer Agreement, PCR primers based on the coxII gene were transferred to the California Dept. of Food and Agriculture for detection of *P. ramorum* and the detection technology is now available to end users such as APHIS.

Documentation:

Martin, F. N., Tooley, P. W., and Blomquist, C. 2004. Molecular detection of *Phytophthora ramorum,* the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. Phytopathology 94:621-631.

Tooley, P. W., Kyde, K. L., and Englander, L. 2004. Susceptibility of selected ericaceous ornamental host species to *Phytophthora ramorum.* Plant Disease 88:993-999.

Soilborne fungal pathogens of alfalfa and dry beans

Accomplishment: A sequence characterized amplified region (SCAR) DNA marker, $OPC7_{1332}$, was developed. The marker can detect the soilborne root rot pathogen *Aphanomyces euteiches* in infected plants and infested field soil. Another SCAR marker ($PSC12_{499}$) was developed; it can detect the soilborne fungus *Phoma sclerotioides*, the causal agent of brown root rot of alfalfa, in infected plants and infested field soil samples. Real-time quantitative PCR assays have been developed that can quantify *A. euteiches* and *Phytophthora medicaginis* in infected alfalfa plants. Tests have been conducted using several different alfalfa populations to determine the correlation between integer disease severity ratings and the amount of pathogen DNA detected in infected plants. The two real-time PCR assays have been used in a multiplex reaction to examine the population

dynamics between *A. euteiches* and *P. medicaginis* in alfalfa plants co-infected with both pathogens.

Impact: The ability to detect the presence of pathogens in fields prior to planting provides growers with decision tools for selecting cultivars that have appropriate disease resistance profiles. At least three weeks are required for detecting *A. euteiches* in soil with conventional microbiological approaches; in the case of *P. sclerotioides*, 60 days are required to unambiguously detect the pathogen in soil samples. The SCAR markers $OPC7_{1332}$ and $PSC12_{499}$ can detect the presence of these pathogens in soil in a single day. The rapid response time for results based on these PCR assays allows for timelier and accurate decision making on the part of growers with respect to choosing cultivars for production in infested fields. The real-time quantitative PCR assay provided a reliable method for distinguishing among commercial alfalfa varieties for resistance to *A. euteiches* based on the analysis of bulked plant samples. Previously, standard tests for evaluating resistance to *A. euteiches* and *P. medicaginis* required the evaluation of many individual plants (≥ 200). The real-time PCR assays will accelerate the process of screening populations by providing a means for determining resistance levels based on an analysis of bulked plant samples. Plants that appear phenotypically similar can be discriminated based on the results of these real-time PCR assays. Commercial seed companies have adopted these technologies to provide additional, more quantitative data to demonstrate the performance of plant populations in support of applications for the review of new varieties. The two real-time PCR assays can be used to simultaneously select plants for resistance to *A. euteiches* and *P. medicagnis*. These assays can also be used to study microbial population dynamics in mixed infections with much greater accuracy and specificity that can be realized using previously employed techniques such as microscopy and enzyme linked immunosorbent assays (ELISA).

Documentation:

Vandemark, G., Barker, B. 2003. Quantifying *Phytophthora medicaginis* in susceptible and resistant alfalfa with a real-time fluorescent PCR assay. Journal of Phytopathology. V. 151:577-583.

Larsen, R., Hollingsworth, C., Vandemark, G., Gritsenko, M., Gray, F. 2002. Use of PCR-based markers for the identification of *Phoma sclerotiodes* causing brown root rot of alfalfa. Plant Disease. V. 86:928-932.

Vandemark, G. J., B. M. Barker and M. A. Gritsenko. 2002. Quantifying *Aphanomyces euteiches* in alfalfa with a fluorescent polymerase chain reaction assay. Phytopathology. V. 92:265-272.

Nematodes

Molecular and Morphological Systematics of Plant-Pathogenic Nematodes:

Accomplishment: ARS researchers developed improved nematode identification techniques and organizational frameworks consisting of comprehensive keys and molecular phylogenetic trees. Species identification keys for the economically important cyst nematode *avenae* group and the complex 111-member stunt nematode group were published. A LSU 28S rDNA phylogenetic tree of lesion nematodes was supplemented with nine more nematode sequences and outgroup taxa to more clearly reflect the expected morphologically based taxonomy. Hsp90, a homologue of the single-copy nematode gene daf-21, was discovered to be a molecular character of potential interest; taxonomically and phylogenetically useful variation was demonstrated in coding and non-coding sequences for several plant-parasitic nematodes. An innovative PCR method led to direct amplification of the Hsp90 gene from single crushed nematodes, and numerous PCR primers and protocols enabled Hsp90 amplification from diverse species of nematodes. A set of protocols for identifying quarantined nematode pests of importance to the North American potato trade was developed. Because accurate identifications can occur only when reliably identified reference specimens are available, the USDA Nematode Collection was maintained, curated, and expanded by adding 5,204 slides and vials from world-wide sources to yield a total collection of 40,079 slides and vials.

Impact: These most current, accurate and inclusive identification keys for stunt and *avenae*-cyst nematodes are used by nematode diagnostic and systematics laboratories throughout the world. The new lesion nematode molecular sequences contributed to the alignment needed for the creation of species-specific primers by other researchers for more timely species identification. Hsp90 is gaining in utility as a new phylogenetic marker for different taxa by researchers in Utah and Italy, who are using the Hsp90 primers and methods. Several novel DNA sequences were submitted to GeneBank. Scientists from the United States, Brazil, and Poland have utilized the novel PCR method. Diagnosticians will utilize the potato nematode identification protocols, thereby minimizing trade disputes involving uncertain nematode identifications. Over 1,224 slides from the USDA Nematode Collection were used by other scientists as reference material, and the Web-based Collection database received over 12,000 hits during the five-year period.

Documentation:

Handoo, Z.A., Carta, L.K., Skantar, A.M. 2001. Morphological and molecular characterization of Pratylenchus arlingtoni n. sp., P. convallariae and P. fallax (Nemata: Pratylenchidae). Nematology. V. 3 p. 607-618.

Handoo, Z.A. 2002. A key and compendium to species of the Heterodera avenae group (Nematode: Heteroderidae). Journal of Nematology. V. 34 p. 250-262.

Skantar, A.M., Carta, L.K. 2000. Amplification of HSP90 homologs from plant-parasitic nematodes using degenerate primers and ramped annealing PCR. Biotechniques: V. 29 p. 1182-1185.

Viruses

Exotic viruses of stone fruits and citrus

Accomplishment: Since the discovery of plum pox(PPV), the most important virus disease of stone fruits, in Pennsylvania in 1999, there has been an effort to develop improved detection capabilities and to understand the pathway of introduction, strain(s) of the virus agent, mechanisms of transmission and the host range. ARS research has established that all isolates found in the United States to date belong to one of two clades of strain D of plum pox potyvirus. A very effective, specific real-time PCR platform was developed for rapid identification of any PPV strain. The potential host range of the virus in wild and ornamental *Prunus* species has been determined, and the role of endemic orchard aphids in local transmission has been established.

Impact: Understanding the molecular nature of PPV populations provides guidance on the origin of the viral introductions, pathways of movement, and frequency of mutational change. Determination of fruit as an infectious unit has resulted in new recommendations for disposal of cull fruit. Determination of the potential host range of the Pennsylvania isolates has reduced the quarantine pressure on movement of cherry germplasm and provided insights into what alternative species may be important sources of inoculum influencing the complete eradication of PPV. The real-time PCR primers and probes developed in our laboratory are being used in the quarantine area in Pennsylvania for rapid, accurate detection of new isolates of PPV.

Documentation:

Schneider, W.L., Sherman, D.J., Stone, A.L., Damsteegt, V.D., and Frederick, R.D. 2004. Specific detection and quantification of Plum pox virus by real-time fluorescent reverse transcription-PCR. Journal of Virological Methods 120:97-105.

Gildow, F., Damsteegt, V., Stone, A., Schneider, W., Luster, D., and Levy, L. 2004. Plum pox in North America: Identification of aphid vectors and a potential role for fruit in virus spread. Phytopathology 94:868-874.

Fatmi, M., Damsteegt, V.D., and Schaad, N.W. 2005. A combined agar-adsorption and BIO-PCR assay for rapid, sensitive detection of *Xylella fastidiosa* in grape and citrus. Plant Pathology 54:1-7.

Viruses of citrus

Accomplishment: Research demonstrated the utility of using degenerate primers and PCR to identify genetically distinct components in complex mixtures of Citrus tristeza virus (CTV).

Impact: These results enhanced regulatory measures concerning identification and management of this pathogen.

Additional information: Major collaborations during this five year cycle have included University of Florida-Citrus Research and Education Center, Florida Department of Agriculture and Consumer Services-Division of Plant Industry and APHIS-PPQ.

Documentation:

Hilf, M.E., V.A. Mavrodieve and S.M. Garnsey. 2005. Genetic Marker Analysis of a Global Collection of Isolates of Citrus tristeza virus: Characterization and Distribution of CTV Genotypes and Association with Symptoms. Phytopathology, in press.

Viruses of floral and woody ornamental plants

Accomplishment: The complete genomes of four unique viruses infecting geranium were determined: Pelargonium line pattern virus, Pelargonium ringspot virus, Elderberry latent virus, and Pelargonium chlorotic ring pattern virus. Nucleic acid probes were developed for detection of each virus. Virus-specific monoclonal antibodies that detect Carnation necrotic fleck virus and Carnation latent virus were developed and transferred to industry (Agdia, Inc) for use in the detection of these important carnation viruses. Single-chain antibody transgene constructs from broad-spectrum reacting potyvirus monoclonal antibody were created and used to transform model plants to test the expression of antiviral antibodies in transgenic plants as a novel means of virus control. Virus-specific and subgroup-specific mouse monoclonal antibodies for the detection and differentiation of isolates of Cucumber mosaic virus (CMV) were produced and are available for public use through Agdia, Inc. A novel method for inoculation of gladiolus with CMV was developed, and the method enables the efficient evaluation of CMV resistance in gladiolus. Several new and emerging viruses were identified with serological and molecular technologies. During an investigation of the influence of temperature on the ultrastructure of Impatiens necrotic spot virus, a high temperature isolate of a tospovirus, Gloxinia HT-1 tospovirus, was recovered and identified from inoculated plants through a series of propagations at high temperature

Impact: This research has developed the tools, reagents and knowledge that will aid U.S. floriculture companies in establishing effective virus testing protocols that will improve clean stock production for new vegetatively-propagated annuals and perennials. Other than natural or engineered resistance (which is rare), the prevention of disease through the development of more effective means for the detection and identification of plant virus diseases affecting ornamentals, and the utilization of those methods to allow selection of pathogen-free or pathogen-indexed plants, is the best method of controlling viral and bacterial diseases. The identification of new and emerging viruses and the availability of reagents for detection will allow growers to test propagation stock in order to select healthy plants, resulting in increased productivity and quality, and customer satisfaction. Improved diagnostic reagents will also help protect domestic crops from existing and foreign pathogens.

Documentation:

Jordan, R.L. and Guaragna, M.A. 2002. Successful development of monoclonal antibodies to three carnation viruses, using an admixture of only partially purified virus

preparations as immunogen, and their use in virus diagnosis. Acta Horticulturae v.568 p.177-184.

Kinard, G.R., Jordan, R.L. 2002. Genome organization of Pelargonium chlorotic ring pattern virus: Further implications for Tombusviridae taxonomy. Acta Horticulturae. v.568 p.17-27.

Zhao, Y., Hammond, J., Tousignant, M.E., Hammond, R.W. 2000. Development and evaluation of a complementation-dependent gene delivery system based on cucumber mosaic virus. Archives of Virology v.145:1-11.

Exotic viruses of sweet potatoes, small fruits (gooseberries and currants) and stone fruits

Accomplishments: Several molecular protocols were developed and used in combination with biological assays for detection of sweet potato viruses, Gooseberry vein banding virus (GVBV), and two cherry flexiviruses in ARS quarantine indexing programs. Detection of viruses in sweet potato by graft inoculating indicator plants takes up to three months. A PCR assay using degenerate primers has been developed and used for the detection of geminiviruses in *in vitro* or *in vivo* plants of *Ipomoea* spp. A real-time PCR procedure for detection of an exotic virus, sweet potato chlorotic stunt virus, was tested and incorporated into the Ipomoea indexing program.

The previous method for detection of GVBV in *Ribes* spp. was based on symptom expression in host plants and graft-inoculation to a bio-indicator, neither of which is reliable. A PCR assay has been adapted and used in routine indexing of the virus of gooseberries and currants.

Graft-inoculation of a woody indicator, Kwanzan flowering cherry, was the only method for detection of Cherry green ring mottle virus (CGRMV) in *Prunus* spp. A one-step RT-PCR assay using consensus primers allows detection of CGRMV and Cherry necrotic rusty mottle virus, a closely related but a previously untargeted flexivirus, in *Prunus* spp.

Impact: Implementation of these molecular methods will improve the reliability and efficiency of the plant quarantine programs. These newly implemented molecular methods will be used as diagnostic tools to identify the target viruses and their variants. Improved detection/diagnostic methods will help exclude exotic pathogens and therefore provide production security for many important commodities.

Additional Information: Collaboration with pathologists at Louisiana State University provided the initial methods for extracting nucleic acid from sweet potatoes and amplifying the geminivirus DNA. The ARS scientists improved the tissue extraction methods, applied the PCR assay to *in vitro* plants, and developed additional primer pairs for detection of sweet potato geminiviruses. Collaboration with LSU was also

instrumental in implementing the real-time PCR assay for sweet potato chlorotic stunt virus.

Documentation:

Salih, S., Waterworth, H., Thompson, D. 2001. Role of plant tissue cultures in international exchange and quarantine of germplasm in the United States and Canada. HortScience. 36: 1015-1021.

Kinard, G.R., Waterworth, H.E., and Mock, R.G. 2001. Advances in quarantine testing of temperate fruit tree germplasm at USDA. Acta Horticulturae. 550: 441-445.

Li, R., Salih, S., Hurtt, S. 2004. Detection of geminiviruses in sweetpotato by polymerase chain reaction. Plant Disease 88: 1347-1351.

Viruses of strawberry, blueberry, blackberry and raspberry

Accomplishment: A new strategy for cloning dsRNA templates was developed and used to efficiently clone viruses from small fruit crops. Cloning was followed by sequencing, developing RT-PCR based laboratory tests for these viruses, and initiating studies on epidemiology. Complete virus sequences were obtained for Strawberry pallidosis, Beet pseudo yellows, Strawberry latent ringspot, Fragaria chiloensis latent virus, Strawberry necrotic shock virus, Blackberry yellow vein, a new closterovirus from raspberry, a new potyvirus from declining blackberry, Black raspberry decline associated virus. Partial sequences were obtained for apple mosaic virus in strawberry, a closterovirus associated with Strawberry chlorotic fleck disease, a new Totivirus from declining blackberry, an insect-like virus from blackberry, a second virus associated with decline in black raspberry, and a virus associated with blueberry fruit drop disease. In addition, five new viruses were identified in mint. Several of these viruses (Strawberry latent ringspot and Tulip virus X have not been reported previously in the United States).

Impact: The sequence information suggested that whiteflies would be the vector of Strawberry pallidosis. This was confirmed by laboratory experiments and has led to the recommendation that strawberry nurseries control whiteflies to prevent the spread of this virus along with Beet pseudo yellows into plant production nurseries. In areas with high whitefly populations, growers now control whiteflies and the result has been a dramatic decrease in strawberry decline in strawberry production fields in California. With the RT-PCR tests we showed that strawberry decline in Oregon, Washington and British Columbia is caused primarily by aphid-borne viruses and that Strawberry crinkle virus previously not found in the region previously is now very common. Growers with fields with high aphid populations are now trying to control them, and early results suggest a reduction in virus decline caused by the aphid transmitted strawberry viruses. These tests have been made available to state departments of agriculture and are being used in the certification program for strawberry plant production in California. Raspberry bushy dwarf resistant red raspberry addresses the most serious disease of red raspberries in the Pacific Northwest.

Additional Information: Funding from commodity groups and cooperation with extension agents has allowed the large scale testing for the strawberry viruses and monitoring the impact of vector control strategies. Development of transgenic raspberries was done in collaboration with Exelixis Plant Sciences Inc. under a CRADA agreement and with funding from the SBIR program. Fruit quality evaluations of transgenic raspberries were done in collaboration with the Food Sciences Department at Oregon State University and were funded by grants from the Northwest Center for Small Fruit Research.

Documentation:

Hankinson, S.C, Martin, R.R., Heflebower, R.F. Jr., Rouse, R., Maas, J. 2000. Survey of strawberry viruses occurring in commercial plantings in the state of Maryland, USA. Advances in Strawberry Research 18:25-32.

Martin, R.R., Mathews, H. 2001. Engineering resistance to *Raspberry Bushy Dwarf Virus.* Acta Hortic. 551:33-37.

Strik, B., Martin, R.R. 2003. Impact of *Raspberry bushy dwarf virus* on 'Marion' blackberry. Plant Dis. 87:294-296.

Tzanetakis, I.E., Martin, R.R. 2004. Complete Nucleotide Sequence of a Strawberry Isolate of *Beet pseudo yellows virus.* Virus Genes 28:239-246.

Tzanetakis, I.E., Keller, K.E., Martin, R.R. 2005. The use of reverse transcriptase for efficient first- and second-strand cDNA synthesis from single- and double-stranded RNA templates, J. Virol. Methods 124:73-77.

Viruses of lettuce

Accomplishment: Lettuce dieback disease is caused by a group of tombusviruses known as either Lettuce necrotic stunt virus (LNSV) or Tomato bushy stunt virus (TBSV). The virus causes severe stunting, necrosis and death of lettuce plants, resulting in serious economic losses in romaine and leaf lettuce production in nearly all lettuce production areas of California and Arizona. ARS research led to the identification of a new tombusvirus species responsible for infection of lettuce and tomato and the development of detection tools for the identification of this virus. In addition, recent studies identified environmental or soil factors that influence disease development; for example, soil salinity has been identified as a factor contributing to elevated incidence of lettuce dieback symptoms when lettuce plants are grown in LNSV-infested soil.

Impact: The new detection tools were critical to the development and release of resistant germplasm by other ARS collaborators. The environmental studies are critical to the management of lettuce dieback disease in infested fields, which are common throughout all production areas in California and Arizona.

Additional Information: This research would not have been possible without the financial support of the California Lettuce Research Board, the California Tomato Commission, and Agdia, Inc. (CRADA).

Documentation:

Grube, R.C., Wintermantel, W.M., Hand P., Aburomia, R., Pink, D.A.C., and Ryder, E.J. 2005. Genetic analysis and mapping of resistance to lettuce dieback, a soilborne disease caused by tombusviruses. *Theoretical and Applied Genetics* 110: 259-268.

Obermeier, C., Sears, J.L., Liu, H.-Y., Schlueter, K.O., Ryder, E.J., Duffus, J.E., Koike, S.T., and Wisler, G.C. 2001. Characterization of distinct tombusviruses that cause diseases of lettuce and tomato in the western United States. *Phytopathology* 91: 797-806.

Multidisciplinary: The Electron Microscopy Unit

Accomplishment: Through the use of low temperature scanning electron microscopy (LTSEM), pests and pathogens were preserved, thereby allowing researchers to observe pest, pathogens, pests, and host-pathogen relationships without the artifact formation associated with traditional fixation methods. Using these procedures, difficult biological specimens of agricultural importance, including soft bodied forms, were studied in natural behavioral positions with results superior in quality to any fixation technique previously described. For example, one specific accoplishment was the discovery that nematode lips and heads differed greatly in appearance from those observed with chemically fixed specimens this yielded more reliable conclusions about their systematics. Scanning or transmission electron microscopic investigations were involved in the identification or characterization of plant disease vectors, other insects and mites, nematodes, parasites, bacteria, food products and other agriculturally important materials, as well as the fine structure of numerous disease-causing agents including *Onchocerca, Cyclospora, Cryptosporidia, Trichoderma, Monacrosporium, Neospora* and *Phytophthora.*

Impact: The widespread impact of this project has allowed for increased resolution of structural features used for accurate identification and the study of the association of organisms with the host material. These methodological improvements have impacted the fields of entomology, acarology, nematology and zoology, allowing researchers to rapidly identify and study organisms of major agricultural importance while maintaining biologically significant associations never before seen during the use of conventional preparative methods.

Additional Information: This multidisciplinary project provided support for research projects within many ARS National Programs (30 collaborators from 16 ARS laboratories) NASA, and the Center for Disease Control. Through the work of a USDA acarologist, mite researchers nationwide utilized LTSEM to image soft bodied forms of insects and mites with clarity never before seen. These investigators provided specimens not widely available for study for use in the testing and documentation of this microscopy method and greatly assisted the work in this field without expenditures to USDA.

Documentation:

Carta L. K., Erbe E. F., Pooley. C., Murphy C. and Wergin W.P., 2003. Chemical fixation/ambient temperature SEM vs cryofixation/low temperature SEM for taxonomic studies of nematodes. Scanning, Vol. 25, (2) pp.54-56 March/April.

Bergin, W.P., E.F. Ere and R. Ochoa. 2001. Versatile and inexpensive specimen holder for high angle azimuth rotation in a low temperature scanning electron microscope. Proc. Micros. Soc. of Am., Microsc. and Microanalysis 7, pp 718-19.

Orion, D., G.Kritzman, SLF. Meyer, E.F. Erbe and D.J. Chitwood. 2001. A role of the gelatinous matrix in the resistance of root-knot nematode (Meloidogyne spp.) eggs to microorganisms. J. of Nematology 33(4):203-207.

Pena, J.E., R. Ochoa, E.F. Erbe. 2002. Polyphagotarsonemus latus (Acari: Tarsonemidae) Research status on Citrus. Proc. Int. Soc. Citriculture 2000. pp. 754-759.

Wergin, W. P., R. W. Yaklich, L. K. Carta, E. F. Erbe and C. A. Murphy. 2000. Effect of an Ice-Nucleating Activity Agent on Subzero Survival of Nematode Juveniles. Journal of Nematology 32(2):198-204.

Discovery Area 4: Contributions to the Development of Regulations Issued by Governmental Agencies

Molecular and Morphological Systematics of Plant-Pathogenic Nematodes

Accomplishment: The wheat seed gall nematode, a nematode of international quarantine importance (not reported in the United States since 1975) was not detected in any wheat samples sent from numerous U.S. locations. However, ARS scientists did discover another devastating seed gall nematode, *Afrina wevelli*, in love grass seeds from South Africa.

Impact: Confirming the absence of the wheat seed gall nematode contributed to the resumption of the $80 million annual wheat trade with Brazil, for which ARS scientists were awarded an Honorable Mention by the Federal Laboratory Consortium for Technology Transfer in 2002. APHIS regulations were implemented against another devastating seed gall nematode, *Afrina wevelli*, in love grass seeds from South Africa.

Additional information: ARS hosted a visit by Brazilian administrators and scientists to jointly inspect and certify that U.S. wheat is seed gall nematode-free; a partnership between ARS, APHIS, and FAS facilitated this visit**.**

Exotic viruses of sweet potatoes

Accomplishment: Scientists may import small quantities of sweet potato germplasm if it undergoes quarantine and indexing for exotic pathogens, primarily viruses and phytoplasmas. An ARS service program is responsible for conducting the quarantine and testing of sweet potato germplasm. A new procedure developed by ARS was shown to be as efficient as the previous, longer quarantine procedure in detecting exotic pathogens that could severely damage U.S. sweet potato production. The new quarantine and indexing program can be completed in only one growing season (6-9 months).

Impact: APHIS approved this modified quarantine and indexing program in 2004, which reduced the quarantine period by more than half. Consequently, the shortened quarantine period for sweet potato will deliver new germplasm to breeders and other recipients in half the time. This germplasm will be used to introduce disease resistance and desirable horticultural traits into new varieties for U.S. markets. This protocol may also serve as a model for sweet potato certification and virus indexing programs.

Biological Control Component

Introduction: Biological control, although environmentally desirable, is often unreliable and difficult to implement. Knowledge of the identity and mode of action of organisms that interfere with pathogens is needed to develop more effective biological control strategies for reduction of crop losses. Knowledge of the ecology of biological control agents, how they affect and are affected by other organisms, and how biological control is influenced by factors such as weather, soil type, conventional fungicide application, and crop variety is needed to make this strategy more effective and reliable. Additionally, information on how fermentation protocols influence biological control agent production, stability, and amenability to formulation must be obtained if the goal of developing more effective biological control products is to be achieved. Additional knowledge is required to identify physical and biologically-based approaches that enhance the performance of biocontrol agents and/or induce host resistance.

Discovery Area 1: Biological Control Foliage and Fruit Diseases

Biological control for cacao and other alternative crops to illicit narcotic crops

Background: Cacao, vegetables, and other crops, which may be alternatives to illicit narcotic crops, are susceptible to many fungal diseases. Among these diseases are those caused by *Phytophthora* spp. (damping-off, root, crown and pod rots, and blights) on cacao and many other crops, and those diseases specifically on cacao caused by *Crinipellis perniciosa* (witches' broom and pod rot) and *Moniliophthora rorei* (frosty pod rot). Successful cultivation of alternative crops is dependent on managing these diseases using integrated disease management strategies, preferably in a sustainable crop production system. Biological control, using antagonistic and parasitic microorganisms indigenous to the areas where the crops are grown, offers a promising approach to disease control, especially in environmentally sensitive areas such as the tropics. Extensive research is being carried out to identify, characterize, and develop biological control and delivery strategies using microorganisms that improve crop health. Mechanisms used by microbes to influence resistance of the crops to plant pathogens are being evaluated and characterized. A systems approach to disease control incorporating biocontrol technology will enable the development of sustainable crop production strategies that will allow management of disease outbreaks and reduction in crop disease losses.

Accomplishment: An epidemiological weather station was designed, constructed and installed in Bahia, Brazil, for the study of beneficial and pathogenic organisms on cacao. This effort included the design, construction and installation of a research platform for conducting epidemiological studies through the biosphere of the cacao tree from the soil to the upper canopy. This equipment has resulted in the completion of experiments to evaluate the behavior of beneficial fungi in controlling witches broom at different parts of the tree in relationship to microclimates.

Impact: Biologists have been studying the performance of commercial biocontrol applications for the control of Witches' Broom in cacao for many years. The data collected has been very variable from season to season and year to year. The weather station that was developed has already yielded results explaining variability of biocontrol performance due to environmental fluctuation over time and within the forest canopy. This data will allow the optimization of formulation and application techniques, as well as selection of the best adapted biocontrol isolates for use in cacao.

Additional Information: Research was carried out through a SCA with Pennsylvania State University.

Biologically-based management of fire blight of apple and pear trees

Background: Fire blight is a serious disease of apple and pear trees caused by the bacterium, *Erwinia amylovora*. The primary infection site is the blossom, where *E. amylovora* becomes established on the stigma during warm weather and can migrate in free moisture and eventually invade through nectary openings. The importance of the disease has increased in the Pacific Northwest due to bacterial resistance to the antibiotic, streptomycin, and the planting of more susceptible apple varieties and rootstocks. Suppression of *E. amylovora* with beneficial bacteria is a relatively new alternative or complementary control strategy. Novel laboratory screening techniques involving the use of detached crab apple flowers led to the discovery of *Pantoea agglomerans* strain E325, which was exceptionally effective in suppressing *E. amylovora* on flower stigmas. A U.S. Patent for the use of strain E325 for fire blight management was issued in July 1999.

Accomplishment: At the beginning of 2000, a patent license agreement and CRADA (extending to August 31, 2004) with Northwest Agricultural Products (NAP) were in place to commercially develop *P. agglomerans* strain E325 for controlling fire blight of apple and pear. In addition to the wild type E325 strain, NAP was provided with derivative antibiotic-resistant strains to monitor populations in the orchard and ensure compatibility with an antibiotic used commercially. This work contributed to the development of a suitable fermentation medium and product formulation by sharing information obtained through laboratory experience, determining the quantity and purity of bacterial cells in NAP samples, and conducting efficacy trials with detached crab apple flowers in the laboratory and on apple trees in the field. Under the name "Bloomtime Biological," the product was field tested in different fruit-producing regions and shown to exceed the efficacy of BlightBan A506, the only biological product currently available for fire blight in the United States. NAP anticipates full EPA registration for the new product sometime in 2005.

Impact: *Pantoea agglomerans* strain E325, marketed as Bloomtime Biological, will serve as an effective tool in the management of fire blight of apple and pear in the United States. It will complement other control strategies and significantly reduce tree and fruit losses attributed to this disease. Efforts to understand the mechanism of strain E325 will

possibly lead to advancements in biological control of fire blight with this or related organisms.

Additional Information: Small grants were received from the Washington Tree Fruit Research Commission to work on biological control of fire blight, and the Winter Pear Control Committee and the Northwest Horticultural Council to conduct research addressing fire blight phytosanitary issues affecting the export of U.S. pears.

Documentation:

Pusey, P.L. 2000. The role of water in epiphytic colonization and infection of pomaceous flowers by *Erwinia amylovora*. Phytopathology 90:1352-1357.

Pusey, P.L. 2002. Biological control agents for fire blight of apple compared under conditions limiting natural dispersal. Plant Dis. 86:639-644.

Pusey, P.L, and Curry, E.A. 2004. Temperature and pomaceous flower age related to colonization by *Erwinia amylovora* and antagonists. Phytopathlogy 94:901-911.

Discovery Area 2: **Biological Control of Soilborne Pathogens**

The microbial and molecular basis of take-all decline

Background: In the United States, wheat is grown on 75-80 million acres of land and barley is grown on 10 million acres. Root diseases, including take-all, *Rhizoctonia*, *Pythium*, and common root rots, and Fusarium foot rot, caused by soilborne fungal pathogens are major production limiting factors in cereal-based cropping systems. Resistance to foliar diseases is common in plants but crop species including wheat and barley lack resistance to many of the most widespread soilborne pathogens. Thus, plants have adopted a strategy of selectively stimulating and supporting populations of antagonistic microorganisms in the rhizosphere environment as the first line of defense against attack by soilborne pathogens. The best example of natural disease suppressiveness is take-all decline (TAD), which is the spontaneous remission in the incidence and severity of take-all during wheat and barley monoculture, following a severe outbreak of the disease. TAD is a global phenomenon and a 1997 survey revealed that many wheat farmers across the United States utilize wheat monoculture to control take-all. Buildup of antagonistic microorganisms in the rhizosphere environment during monoculture was thought to be responsible for TAD, but the exact microbial and molecular basis of the suppressiveness remained a mystery.

Accomplishment: It was demonstrated that TAD results from the buildup in the rhizosphere environment of fluorescent *Pseudomonas* spp., producing the antibiotic 2,4-diacetylphloroglucinol (DAPG). Genetic probes and primers specific for *phlD*, a key gene in the DAPG biosynthetic locus, in combination with colony hybridization and PCR, were used to demonstrate that DAPG producers established threshold population densities of $>10^5$ DAPG producers/gram of root (required for disease suppression) in

TAD soils but remain below the threshold population on wheat grown in non-suppressive soils. Transfer of TAD soil to non-TAD (conducive) soil established threshold densities of DAPG producers and suppressiveness, and reduction of DAPG producers below the threshold eliminated suppressiveness. DAPG was isolated from roots grown in TAD but not conducive soils. It was shown that enrichment of DAPG producers by wheat monoculture was a common phenomenon throughout the United States, including in Fargo, North Dakota on the North Dakota State University continuous spring wheat plot No. 2, established in the 1882 by W. M. Hayes and listed in the National Register of Historic Places. DAPG-producing fluorescent *Pseudomonas* spp. were shown to be responsible for TAD in Dutch soils. Repetitive-sequence based (rep) PCR and RFLP and sequence analysis of *phlD* identified 18 distinct genotypes (A–Q & T) of DAPG producers, however, in Washington TAD soils D-genotype isolates are primarily responsible for disease suppression because of their unique affinity for wheat roots. Introduction of very small doses of D-genotype isolates into conducive soils duplicates TAD and the bacteria remain at threshold densities in the soil as long as wheat is grown.

Impact: TAD soils were described over 60 years ago and are used throughout the world to control take-all, one of the most important root diseases of wheat. These studies are the first to describe the molecular basis of take-all decline or any suppressive soil, and ended 50 years of debate about the biological basis of TAD. These findings have rejuvenated research on suppressive soils by plant pathologists and microbiologists using molecular biology approaches, and have resulted in greater awareness of the potential of suppressive soils to control soilborne pathogens in sustainable farming systems. As a result of these studies, DAPG producers are now recognized as playing key roles in plant defense of other crop species grown in monoculture. This technology has been patented and D-genotype strains are under test for commercial development.

Additional Information: The research was conducted in collaboration with the Department of Plant Sciences, Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands. Competitive grants supported research that contributed to this accomplishment and NRI.

Documentation:

McSpadden Gardener, B. B., Schroeder, K. L., Kalloger, S. E., Raaijmakers, J. M., Thomashow, L. S., and Weller, D. M. 2000. Genotypic and phenotypic diversity of *phlD*-containing *Pseudomonas* isolated from the rhizosphere of wheat. Applied and Environmental Microbiology. V. 66, p. 1939-1946.

Raaijmakers, J. M., and Weller, D. M. 2001. Exploiting genotypic diversity of 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp.: Characterization of superior root-colonizing *P. fluorescens* strain Q8r1-96. Applied and Environmental Microbiology. V. 67, p. 2545-2554.

Weller, D. M., Raaijmakers, J. M., McSpadden Gardener, B. B., and Thomashow, L. S. 2002. Microbial populations responsible for specific suppressiveness to plant pathogens. Annual Review of Phytopathology. V. 40, p. 309-348.

de Souza, J. T., Weller, D. M., and Raaijmakers, J. M. 2002. Frequency, diversity, and activity of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in Dutch take-all decline soils. Phytopathology. V. 93, p. 54-63.

Landa, B. B., Mavrodi, D. M., Thomshow, L. S., and Weller, D. M. 2003. Interactions between strains of 2,4-diacetylphloroglucingo-producing *Pseudomonas fluorescens* in the rhizosphere of wheat. Phytopathology. V. 93, p. 982-994.

Biological control of soilborne pathogens of potato in New England cropping systems

Background: Agricultural production in the New England Region has seriously declined in recent years. Within the past decade alone, farmland in New England has decreased by 393,000 acres and potato production, once a major rotation crop in New England agriculture, has seriously declined because of competition from other potato growing regions in the United States and diseases. One of the most important diseases in the New England region is Late Blight (*Phytophthora infestans),* which causes significant losses to the potato and tomato industries every year. Sustainable cropping systems and disease management practices are needed that rely on biological control and other natural methods of disease control.

Accomplishment: Potatoes suffer from numerous plant diseases, and billions of dollars are spent annually in the United States to control these diseases. Sustainable methods of disease control were evaluated that include rotations, green manures, cover crops, and organic amendments, along with biocontrol agents, microbial inoculants, biostimulants, and novel biocontrol organisms. Commercially-available biological control organisms such as *Trichoderma virens* and *Bacillus subtilis* reduced Rhizoctonia stem canker by 37-75% and black scurf by 11-20%, while increasing potato yield by 15-20%. These practices/amendments have significant potential for improving disease control and reducing pesticide inputs.

Impact: The development and transfer of sustainable biocontrol technologies to New England growers has helped improve agricultural viability and rural economic vitality in the Northeast.

Additional Information: The Maine Potato Board has contributed funding to evaluate potential controls for powdery scab. This has facilitated performing on-farm research that has identified the potential of *Brassica* crops for controlling powdery scab and other soil-born diseases.

Documentation:

Larkin, R.P. 2003. Characterization of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles. Soil Biology & Biochemistry 35:1451-1466.

Biology and management of soilborne diseases and beneficial soil and root-inhabiting microorganisms in nursery and other horticultural crops

Background: Soilborne diseases of nursery and other horticultural crops cause serious losses to growers in the United States and throughout the world. Control or management strategies are limited at present, with growers relying heavily on chemical agents to eliminate or suppress these diseases. One such approach is preplant fumigation of the soil in nurseries or other plantings to eliminate or greatly reduce pathogen populations in the soil, but that approach has significant risks to workers and to the environment. This research has been largely focused on finding alternative approaches to controlling such pathogens, especially biological and cultural methods. This work has emphasized the roles played by beneficial microorganisms in the rhizosphere soil of crop plants, especially rhizobacteria and mycorrhizal fungi and has emphasized those cultural practices that most affect the establishment and function of mycorrhizal fungi and bacterial biocontrol agents within the production system.

Accomplishments: Mycorrhizal fungi are beneficial fungi that form a symbiotic relationship with most plants on Earth. Much of the nursery industry employs soilless plant growth media in most or all of the production systems. Mycorrhizal fungi are inhibited by many of the cultural practices commonly used, especially heavy use of fertilizers and pesticides. In a series of studies many of those practices, especially the media components and the application of fertilizers have been addressed. The works has shown that formation and function of mycorrhizae in soilless media can be affected adversely by some peat mosses used, by some forms of organic or inorganic fertilizers (especially with high P content), and by most composts. These studies were reported in a series of papers in the horticultural journals. Currently, these studies have shown that formation of arbuscular mycorrhizae stimulates an increase in population of antagonistic rhizobacteria in rhizosphere soil, thus indicating a role for these fungal symbionts in the biological control of soilborne fungal pathogens.

Impact: Understanding the factors in usual production practices of nursery crops that might favor the establishment of beneficial microbes, such as mycorrhizal fungi and antagonistic rhizobacteria is key to modifying practices to accommodate these beneficial organisms as a means of suppressing soilborne pathogens. Growers now have some guidance as to how to favor beneficials as a means of controlling soilborne pathogens rather than relying strictly on the application of chemical agents.

Documentation:

Linderman, R. G., Davis, E. A. 2004. Evaluation of commercial inorganic and organic fertilizer effects on arbuscular mycorrhizae formed by *Glomus intraradices*. HortTechnology 14(2): p. 196-202.

Linderman, R. G., Davis, E. A. 2005. Comparative plant susceptibility and *Phytophthora* species' virulence on detached nursery crop leaves. Submitted to journal, in revision.

Brodhagen, M., Henkels, M. D., and Loper, J. E. 2004. Positive autoregulation of the antibiotic pyoluteorin in the biological control organism *Pseudomonas fluorescens* Pf-5. Appl. Environ. Microbiol. 70:1758-1766.

Stockwell, V. O., Johnson, K. B., and Loper, J. E. 2002. Antibiosis contributes to biological control of fire blight by *Pantoea agglomerans* strain Eh252 in the field. Phytopathology 92: 1202-1209.

Anderson, L.M., Stockwell, V.O., and Loper, J.E. 2004. An extracellular protease of *Pseudomonas fluorescens* A506 inactivates antibiotics of *Pantoea agglomerans*. Phytopathology 94:1228-1234.

Pinkerton, J. N., Schreiner, R. P., Ivors, K. L., Vasconcelos, M. C. 2004. Effects of *Mesocriconema xenoplax* on *Vitis vinifera* and associated mycorrhizal fungi. Journal of Nematology 36:193-201.

Critical assessment of compost teas for disease control

Background: There has been increased popular interest in the utilization of compost teas to help improve plant health. However, there are few critical evaluations of their utility for disease control despite numerous popular claims. In addition, within the popular press there were numerous claims that were contradictory to the current scientific evidence. We endeavored to systematically evaluate the utility of compost teas and numerous tea production methods for control of plant diseases.

Accomplishment: It was demonstrated that popular press claims that aerobically produced compost teas (requiring expensive equipment) were superior to nonaerated (i.e., anaerobic) compost teas in controlling foliar and soilborne diseases were misleading and that nonaerated compost teas did not cause phytotoxicity as is commonly claimed. It was also established that compost source was more important than the tea production method in obtaining disease suppressive compost tea. The consistency of disease control was increased through the addition of spreaders and stickers that increased the distribution of microbes on the leaf surface.

Impact: This research generated critical knowledge and addressed numerous misconceptions so that practitioners choosing compost teas as a disease management tool now have a solid foundation from which to make decisions. This research is particularly beneficial to organic growers since they have few options for disease management.

Additional Information: The research on Compost Tea was conducted in collaboration with Oregon State University. Outside funding came from Oregon Department of Agriculture and the Oregon Nurserymen Association.

Documentation:

Scheuerell, S.J. Sullivan, D. and Mahaffee, W.F. 2005. Suppression of Seedling Damping-Off Caused by *Pythium ultimum*, *Pythium irregulare*, and *Rhizoctonia solani* in Compost Amended Container Media. Phytopathology 95:306-315.

Scheuerell, S.J. and Mahaffee, W.F. 2004. Compost Tea as a Container Media Drench for Suppressing Seedling Damping-Off Caused by *Pythium ultimum*. Phytopathology 94:1156-1163

Antagonistic microbes for management of plant-parasitic nematodes

Background: Plant-parasitic nematodes are microscopic soil worms that reduce the yield and quality of many crops. Nematodes cause nearly ten billion dollars in crop losses per year in the United States and nearly 100 billion dollars globally. For some crops, such as soybeans, nematodes are the most important pest or pathogen. However, many of the conventional nematicides that were used to control plant-parasitic nematodes have been shown to contribute to groundwater contamination and to be hazardous to the health of humans and animals, and have therefore been banned or restricted in use. It is particularly notable that the restrictions on methyl bromide application will remove the most widely used nematicide from agricultural use. Consequently, new, effective and environmentally safe tools for managing nematodes are urgently needed. ARS is developing nematode management systems based upon three different strategies: 1) determining the usefulness of antagonistic fungi and bacteria (alone and in combination with other organisms or practices), 2) identifying natural products that adversely affect nematodes, and 3) applying soil amendments to reduce nematode crop losses.

Accomplishment: (1) Nematode-Antagonistic Microbes. Biocontrol agents that are active against multiple pathogens or pests are more likely to be cost-effective in commercial formulations than organisms that are only active against a single target. ARS Scientists and collaborators tested fungi and bacteria for production of compounds toxic to root-knot nematodes (RKN; *Meloidogyne* spp.), and for ability to act as biocontrol agents against RKN populations on pepper and cucurbit roots. Many of the tested microbes had already been shown to be active against plant-pathogenic fungi. Two of the tested microbes, the fungus *Trichoderma virens* and the bacterium *Burkholderia cepacia*, were isolates of species that are sold commercially for management of soilborne pathogens. However, the ability of strains in the ARS collection to control nematodes was poorly known, and research demonstrated that the tested strains of *Burkholderia* and *Trichoderma* could suppress nematode populations on bell pepper.

Impact: (1) Nematode-Antagonistic Microbes. This research contributed to the establishment of a collaborative agreement entitled "Development of Biological Controls for Suppression of Select Soilborne Diseases of Cucumber and Other Greenhouse-grown Vegetables" with a private sector firm; a ARS scientist was involved in the work on this project. The microbes that were identified as active against nematodes could potentially be applied by growers in an integrated pest management system for reduction of certain plant diseases caused by fungi and nematodes.

Accomplishment: (2) Natural products. One potential means of managing plant-parasitic nematodes is by soil application of natural products as alternatives to fumigant and non-fumigant nematicides. To obtain active natural products for management of soybean cyst nematode (SCN) and other plant-parasitic nematodes, fungi were isolated from SCN on soybean plants in China, the geographic location where SCN and its host plant (soybean) originated. ARS scientists and collaborators isolated the fungi, imported the isolates to the United States, and identified the fungal isolates. The imported fungi (254 isolates) were assayed for production of compounds that increased or reduced nematode egg hatch, or that affected mobility of hatched juvenile nematodes; assays were conducted with SCN and RKN. The active natural products were isolated and identified from two fungal species, and one compound was chemically synthesized.

Impact: (2) Natural Products. Scientists in the United States now have many fungi from China for evaluation in biocontrol programs; this is particularly important because this is the only research being conducted on imported exotic microbes for SCN management. In addition, the developed assay successfully identified active natural compounds produced by nematode-associated fungi, and the natural products are being studied for biologically based management. The research clearly confirmed that target nematode species must be used in assays for bioactive products, because compounds active against one nematode were not necessarily active against another. This is significant because researchers frequently use bacterial-feeding nematodes (which are generally easier to culture than plant-parasites) when looking for active compounds to apply against plant-parasitic nematodes. Natural products, when appropriately delivered to plants, may be used by growers to reduce crop losses caused by plant-parasitic nematodes.

Additional Information: Researchers from ARS collaborated with scientists at the following locations: 1) Institute of Biological Control (Chinese Academy of Sciences, Beijing, China) for collecting fungi from China; 2) Fusarium Research Center (Pennsylvania State University) for fungal identifications; 3) University of MD for identifying and synthesizing fungal compounds toxic to nematodes.

Documentation:

Meyer, S.L.F., R.N. Huettel, X.-Z. Liu, R.A. Humber, J. Juba, and J. Nitao. 2004. Activity of fungal culture filtrates against soybean cyst nematode and root-knot nematode egg hatch and juvenile mobility. Nematology 6:23-32.

Zasada, I.A. and M. Tenuta. 2004. Chemical-mediated toxicity of N-Viro Soil to *Heterodera glycines* and *Meloidogyne incognita*. Journal of Nematology 36:297-302,

Chitwood, D.J. 2002. Phytochemical based strategies for nematode control. Annual Review of Phytopathology 40:221-249.

Nitao, J.K., S.L.F. Meyer, W.F. Schmidt, J.C. Fettinger, and D.J. Chitwood. 2001. Nematode-antagonistic trichothecenes from *Fusarium equiseti*. Journal of Chemical Ecology 27:859-869.

Meyer, S.L.F., S.I. Massoud, D.J. Chitwood, and D.P. Roberts. 2000. Evaluation of *Trichoderma virens* and *Burkholderia cepacia* for antagonistic activity against root-knot nematode, *Meloidogyne incognita*. Nematology 2:871-879.

Discovery Area 3: Biocontrol of Postharvest Diseases

Microbial production and formulation technologies for biocontrol of postharvest diseases of potato

Background: Eighty percent of pathogen strains causing Fusarium dry rot of stored potato tubers are now genetically resistant to thiabendazole, the only fungicide registered for postharvest use. Pseudomonads and other Gram-negative bacterial strains capable of dry rot suppression were identified in soil samples from fields with low disease incidence and ranked on the basis of growth kinetics in liquid culture and subsequent efficacy when delivered to wounded potatoes challenged with pathogen. Subsequent studies indicated that all of the dry rot antagonists produced at least one, and often multiple antifungal compounds active against the pathogen *Fusarium sambucinum.* Plant growth regulating compounds are also known to be produced by many pseudomonads. Liquid culture nutrition was shown to influence the accumulations of bioactive metabolites and impact biocontrol of dry rot as well as other potato storage diseases and sprouting. Therefore studies elucidating biocontrol agent metabolite bioactivities, dependence of metabolism on culture conditions, and target pest range are critical to enhancing commercial potential.

Accomplishment: Biological control strains and production/formulation protocols for biological control treatments capable of protecting postharvest potatoes in storage from dry rot, sprouting and late blight was discovered. Discoveries leading to this accomplishment included: the finding that several of our top dry rot antagonists suppressed sprouting in the laboratory and then three strains were able to inhibit sprouting in pilot trials for 4-5 months at a level similar to the chemical inhibitor 16 ppm CIPC thermal fog; observations that biocontrol success by any given bacterial strain was dependent on production culture nutrients, cultivar and pathogen strain; the discovery that the plant growth hormone indoleacetic acid (IAA) had antifungal activity against dry rot and that cultures of two bacteria strains could accumulate IAA in concentrations sufficient to cause sprout suppression when provided optimal nutrients in culture; the discovery that applying mixtures of top biocontrol strains was not detrimental and often enhanced efficacy and consistency of control from trial to trial; the discovery that bacterial antagonists could retain 60-90% of original population after 24 hours in the presence of one quarter strength CIPC sprout inhibitor and that the CIPC application rate needed for sprout control could be reduced by 75 to 90% if used in combination with the biological control strains; the result that late blight suppressiveness was indicated for several of our top dry rot suppressive strains in a laboratory screening of strains x production media x formulation; and the finding that top dry rot antagonistic bacteria were able to significantly suppress late blight infections by 20 to 90% the first year and 35 to 91% in the second season of pilot testing.

Impact: This accomplishment allows biocontrol of multiple potato storage problems including dry rot, late blight, and sprouting of stored potatoes with a single biocontrol treatment sprayed to potatoes as they enter storage, much like the traditional application

of the now ineffective chemical TBZ for control of fungal diseases of tubers in storage. The development of commercial biocontrol agents to address these postharvest storage problems could save U.S. potato growers $100-500 million per year in crop losses due to dry rot and late blight diseases alone. Sprout control, required for 50% of the potato crop, is an added benefit of our disease suppressive biocontrol agents, and may allow reduced use of CIPC which faces tightening EPA regulation even though it is the only sprout inhibitor approved for postharvest use in the United States. Multiple strains were patented as biological control agents against dry rot [Slininger, Schisler, and Bothast (1996), US Patent 5,552,315; Schisler, Bothast, and Slininger (1998), US Patent 5,783,411] and, during this review period, sprouting [Slininger, Burkhead, Schisler, and Bothast (2000), U.S Patent 6,107,247].

Additional Information: Collaboration with researchers at Mercer University, Macon, Georgia, led to the isolation of a compound from a culture of dry rot antagonistic bacteria which stimulated sprout suppressive bioactivity in the presence of the bacteria and which also exhibited dry rot suppressive bioactivity. The compound has now also been shown to reduce the development of late blight caused by *Phytophthora infestans* infection of stored potatoes. A simple derivative of this natural product applied at relatively low dosage has exhibited the ability to nearly completely inhibit the growth of *P. infestans* on plates and to suppress the development of late blight symptoms in stored potatoes.

A current Cooperative Research and Development Agreement with an agricultural company located in the Pacific Northwest is partially funding pilot testing of our biological agents for control of dry rot, sprouting and late blight and enabling the company to make a licensing decision regarding our technology. The University of Idaho Kimberly Research Station has provided expert assistance in designing potato storage experiments and has been the site of our annual pilot experiments to test new biocontrol agent formulations and activities discovered in laboratory experiments. These collaborations have given us invaluable exposure to real world problems and practices in the potato industry, as well as large-scale fermentation techniques, traditional formulation techniques, marketing considerations, and commercialization requirements that must be met in order to realize a biocontrol product for use on postharvest potatoes.

Documentation:

Schisler, D.A., Slininger, P.J., Hanson, L.E., Loria, R. 2000. Potato cultivar, pathogen isolate, and antagonist cultivation medium influence the efficacy and ranking of biological control strains of Fusarium dry rot. Biocontrol Science and Technology 10:267-279.

Slininger, P.J., Schisler, D.A., Burkhead, K.D., Bothast, R.J. 2003. Postharvest biological control of potato sprouting by dry rot antagonistic bacteria. Biocontrol Science and Technology 13(5):477-494.

Slininger, P.J., Behle, R.W., Jackson, M.A., Schisler, D.A. 2003. Forum: Discovery and development of biological agents to control crop pests. Neotropical Entomology 32(2):183-195.

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Slininger, P.J., Burkhead, K.D., and Schisler, D.A. 2004. Antifungal and sprout regulatory bioactivities of phenylacetic acid, indole-3-acetic acid, and tyrosol isolated from the potato dry rot suppressive bacterium *Enterobacter cloacae* S11:T:07. Journal of Industrial Microbiology and Biotechnology 31:517-524.

Emerging technologies to control postharvest diseases of citrus

Background: Fresh fruit are attacked by fungal diseases that rot fruit after harvest. The fungal diseases that rot fruit after harvest cause losses of about 5% of California's $3 billion production. The control of the disease-causing fungi is important, and is usually accomplished using chemical fungicides and sanitizers, but issues of pest resistance to these chemicals, the dietary safety of their presence foods, and their impact on the environment has made the search for safer replacements important. Fungicides used to control these diseases interfere with the export of citrus fruit because some countries will not accept residues of the fungicides on the fruit. Three fungicides are approved for citrus fruit use in California, sodium ortho-phenyl phenate, imazalil, and thiabendazole. Sodium ortho-phenyl phenate is classified as a probable human carcinogen and imazalil as a possible human carcinogen by the U.S. Environmental Protection Agency.

Accomplishment: Effective combinations of a biological control bacterium (*Pantoea agglomerans*) and postharvest thermal regimes were developed to control postharvest decay of citrus fruit. This technique reduces fungicide costs, decay losses by packers, and fungicide residues in citrus products purchased by consumers. In a series of experiments to establish optimum regimes, citrus fruit to which the bacterium had been applied were placed under warm, humid storage for up to 3 days at up to 44°C.

Impact: This project investigated one strategy to manage and minimize postharvest decay losses of citrus fruit without fungicides, the integrated combination of citrus postharvest thermal treatment and a well-studied biological control antagonist. This approach is novel and not yet in commercial use, although pilot-scale demonstration experiments are now in progress in California and Spain.

Additional Information: Supplemental funds for this work were contributed by the California Citrus Research Board. Much of this work was done in collaboration with a visiting scientist of the IRTA, University of Lerida, Lerida, Spain. The collaboration was funded entirely by grants from the Spanish citrus industry and Catalonian government.

Documentation:

Plaza, P., Usall, J., Smilanick, J. L., Lamarca, N. and Viñas, I. 2004. Combining *Pantoea agglomerans* (CPA-2) and curing treatments to control established infections of *Penicillium digitatum* on lemons. J. Food Protection V. 67 p. 781-786.

Plaza, P., Usall, J., Torres, R., Abadias, M., Smilanick, J. L., and Viñas, I. 2004. The use of sodium carbonate to improve curing treatments against green and blue moulds on citrus fruits. Pest Management Science V. 60 p. 815-821.

Smilanick, J. L., Sorenson, D., Mansour, M., Aieyabei, J., and Plaza, P. 2003. Impact of a brief postharvest hot water drench treatment on decay, fruit appearance, and microbe populations of California lemons and oranges. HortTechnology V. 13 p. 333-338.

Palou, L., Smilanick, J. L., Crisosto, C. H., Mansour, M., and Plaza, P. 2003. Ozone gas penetration and control of the sporulation of *Penicillium digitatum* and *Penicillium italicum* within commercial packages of oranges during cold storage. Crop Protection V. 22 p. 1131-1134.

El-Ghaouth, A.; Smilanick, J. L.; Brown, G. E.; Ippolito, A.; Wilson, C. L. 2001. Control of decay of apple and citrus fruits in semi-commercial tests with *Candida saitoana* and 2-deoxy-D-glucose. Biological Control V. 20 p. 96-101. 2001.

Biological control of postharvest diseases of deciduous fruit trees

Background: When taking into account the wide array of conditions present in packinghouse facilities (different cultivars, different levels of fruit maturity, different levels of inoculum pressure, etc.), the performance of postharvest biological control agents is generally more variable than for chemical fungicides. For example, as fruit mature, higher concentrations of an antagonist must be used to achieve the same level of decay control as on less mature fruits. Additionally, current postharvest biocontrol products are only protective and do not exhibit eradicant activity. Therefore, the efficacy and performance reliability for biocontrol must be increased. This is being accomplished through ARS studies on the mode of action of postharvest biocontrol agents, combining the use of biocontrol agents with natural compounds and/or heat treatments, and genetic engineering.

Accomplishments: ARS scientists at Kearneysville, West Virginia, discovered and patented two biocontrol agents that were commercialized as the products, Aspire and Bio-Save, through a CRADA with industry. Subsequently, ARS scientists have found that the efficacy of yeast antagonists can be greatly enhanced by the addition of natural, bioactive compounds. This technology has been patented and is being commercialized via a CRADA with industry as a “bioactive coating@ that can serve as an effective alternative to synthetic fungicides for the control of postharvest diseases of pome and citrus fruit.

A laboratory screen was devised that allowed the discovery of a number of natural volatile fragrance/flavor compounds and anti-microbial compounds to two major food

contaminating bacteria (*Escherichia coli* and *Pseudomonas marginalis*) and two mold fungi (*Botrytis cinerea* and *Penicillium expansum*). When these natural anti-microbial compounds are released in the headspace of plastic bags containing fruit and vegetables they extend the shelf life of these commodities. A new "active" plastic food storage bag design was invented that responds to moisture released from commodities stored in it by releasing carbon dioxide. The increased carbon dioxide concentration in the bag reduces microbial growth and extends the shelf life of the stored commodity. Natural anti-microbial volatile compounds can also be used at lower concentrations when they are combined with this "active" carbon dioxide generating technology.

In collaboration with USDA-ARS scientists at Beltsville, Maryland a new strategy for integrated fruit decay control was developed. This strategy combines heat treatments with hot air (4d at 38C), and sodium bicarbonate with biological control by bacterial and heat tolerant yeast antagonists. Combination of treatments also resulted in better control than either treatment alone. These treatments complemented each other in that heat provided eradicative activity up to 24 h after inoculation with *P. expansum*, and biocontrol agents provided residual activity. The heat or sodium bicarbonate treatments alone can not provide adequate decay control by themselves, but biological control combined with those methods provided decay control that is broader in the spectrum of activity and more efficient than the individual treatments. A heat tolerant yeast, *Metschnikowia pulcherrima*, isolated from wounded apple in an unmanaged orchard was used. This yeast is a common inhabitant of fruit including apple and grape, is commonly found in apple cider and is an integral part in the wine-making process. It was found that the use of 1-MCP on harvested fruit to inhibit maturation can predispose fruit to decay, but the alternatives to fungicides are capable of preventing this increase in decay.

A phage treatment was found to be very effective in reducing populations of *Listeria moncytogenes* on fresh cut melon. The most effective were cocktail of phages at concentrations around 10^8 PFU/ml and when the application was up to 1h before contamination of the fruit. This implies that under commercial conditions the phage would have to be applied at the time of fruit cutting or shortly thereafter. It was found that a bacterium, that commonly occurs in fruit juices, and a biocontrol yeast were very effective in controlling *L. monocytogenes* and *Salmonella* Poona on fresh cut apples. Use of the phage cocktail in combination with these biocontrol agents for control of *L. monocytogenes* and *Salmonella* Poona on fresh cut fruits is currently being explored.

Impact: The development of potential products described above with increased efficacy and the ability to eradicate pre-existing infections will provide a new approach to postharvest disease control. Additionally, the research has extended the potential application of biological approaches to disease control to the consumer. While still in the development phase, industry has shown a great interest in using this technology in plastic storage bags. This research demonstrated that the biological control of postharvest diseases can be easily integrated with other methods with additive and synergistic effects, which also broaden the spectrum of activity (eradicative effect of heat) and further advanced the goal toward eliminating fungicide treatment on fruits after harvest. The

work provided recommendations for the future integration of biocontrol with the other treatments.

Additional Information: Research on the development of bioactive coatings was supported through a CRADA with industry over a 5 year period and the work on active packaging was also supported through a CRADA with industry. Fundamental research on the role of lytic peptides on biocontrol efficacy and genetic engineering was supported by the Bi-National Agricultural Research and Development Fund (BARD).

Documentation:

Bassett, C.L., and Janisiewicz, W.J. 2003. Electroporation and stable maintenance of plasmid DNAs in a biocontrol strain of *Pseudomonas syringae*. Biotechnology Letters 25:199-203.

Janisiewicz, W. J., Leverentz, B, Conway, W. S., Saftner, R. A., Reed, A. N., and Camp, M. J. 2003. Control of bitter rot and blue mold of apples by integrating heat and antagonist treatments on 1-mcp treated fruit stored under controlled atmosphere conditions. Postharvest Biology and Technology. 29: 129-143.

Norelli, J. L., and Miller, S. L. 2004. Effect of prohexadione-calcium dose level on shoot growth and fire blight in young apple trees. Plant Disease 88:1099-1106.

Wilson, C. L. and Peach, P.A. Docket No. 0013.03. "Compartmentalized Plastic Containers for the Segregation and Delivery of Compounds/Carriers in Active Packaging." (11/12/2002).

Wisniewski, M., Bassett, C.L., Artlip, T., Webb, R., Janisiewicz, W., Norelli, J., Goldway, M., and Droby, S. 2003. Characterization of a defensin in bark and fruit tissues of peach and antimicrobial activity of a recombinant defensin in the yeast, *Pichia guilliermondii*. Physiologia Plantarum. 119: 563-572.

Discovery Area 4: Culturing, Formulation and Application Technologies for Biocontrol Agents

Culture and formulation technologies and protocols to develop more effective biocontrol agents

Background: Thousands of potential microbial biocontrol agents have been discovered but fewer than 100 (40 antagonists of plant diseases) have reached the commercial production stage. A major obstacle to biocontrol agent commercialization has been the lack of basic knowledge and experience with methodologies, techniques and tools for manufacturing, storage and delivery of cost effective products.

Accomplishment: We have discovered numerous culture and formulation protocols influencing the abilities of biocontrol strains to tolerate stress factors encountered during

production and formulation. Elements of this accomplishment included: development of a microplate-based high throughput screening technique to rapidly identify optimal cultivation, formulation, drying, and storage protocols that yield active biocontrol agents; identification of proline as an amino acid important to metabolite regulation and enhanced production of viable pseudomonads resistant to toxic culture endproducts; identification of di- and tri-saccharide sugars such as lactose, turanose and melezitose as stabilizers during rapid drying of gram-negative bacteria; identification of relative humidity and sugar content requirements for long term survival of pseudomonads in dry storage; discovery of the optimal carbon to nitrogen ratio and carbon loading for cell cultivation and also a unique cold shock protocol to finish the cultivation and induce enhanced dry storage survival of a wheat scab suppressive yeast.

Impact: These discoveries are key to the commercial feasibility of applying beneficial microbial agents for suppressing take-all and Fusarium head blight of wheat as well as dry rot, late blight and sprouting of postharvest potatoes in storage. The development of commercial biocontrol agents to address these field and storage problems could save U.S. wheat growers up to $1.0 billion per year and potato growers $100-500 million per year in crop losses due to fungal disease. Sprout control, required for 50% of the potato crop, is an added benefit of our disease suppressive biocontrol agents, and may allow reduced use of CIPC which faces tightening EPA regulation even though it is the only sprout inhibitor approved for postharvest use in the United States. Numerous invited presentations and publications of these results by ARS scientists have convinced many scientists of the critical importance of optimizing the fermentation environment to enhance the amenability of biomass products to stresses encountered during formulation and stabilization.

Additional Information: Collaboration with a Florida-based company provided evidence that pseudomonads could survive with less than 50% loss of viable units on dry nutrient granules for several months at 25^{o}C and could be used to reduce the chemical fertilizer needs of turf establishment. This discovery has lead to more controlled experiments to determine cell survival requirements and to design granule composition to control cell survival to make this technique more generally applicable to the drying and storage of gram-negative bacteria for use in many areas of biological control.

In preparation of development of a new DNA oligo microarray for *Pseudomonas fluorescens* Pf-5, a plant disease biocontrol agent, a set of quality controls using exogenous nucleic acids was generated. The reliability and reproducibility of microarray data are critical for obtaining meaningful biological insights from genomic expression analysis. Quality control of microarray experiments is an important element for data handling of microarray experiments. These quality controls were developed in defined concentrations and are ready for incorporation into each RNA labeling reaction. Due to the uniqueness of these selected control genes, our quality controls can be used as universal controls for any microbial DNA oligo microarrays, as well as for a wide range of microarrays for plant and animals. In collaboration with ARS scientists in Corvallis, Oregon a DNA oligo microarray of the recently sequenced *Pseudomonas fluorescens* Pf-5 genome has been developed and printed. Microarray analysis of Pf-5 genome will be

used to study stress tolerance mechanisms of pseudomonads and how they can be regulated and controlled via cell cultivation and formulation techniques. In recognition for this work, an ARS scientist has been invited to represent ARS as a member of the External RNA Control Consortium (organized and sponsored by the Biotechnology Division, National Institute of Standards and Technology) to establish universal control protocols for the collection of microarray data from diverse genomes and laboratories.

Documentation:

Slininger, P.J., Schisler, D.A. 2003. High throughput assay for optimizing microbial biological control agent production and delivery. Phytopathology. 93(6):S79 [2003 Poster Proceedings of The American Phytopathological Society, August 9-13, Charlotte, NC (on compact disk)].

Schisler, D.A., Slininger, P.J., Behle, R.W., and Jackson, M.A. 2004. Formulation of *Bacillus* spp. for biological control of plant diseases. Phytopathology. 94(11):1267-1271.

Zhang, S., Schisler, D.A., Jackson, M.A., Boehm, M.J., Slininger, P.J. 2004. USDA-ARS, Ohio State University cooperative research on biological control of Fusarium head blight 2: Cold temperature shock during production of Cryptococcus nodaensis OH 182.9 enhances cell survival after air-drying. In: Proceedings of the 2nd International Symposium on Fusarium Head Blight Vol. 2, December 11-15, 2004, Orlando, FL pp. 383-387.

Slininger, P.J., and Shea-Andersh, M.A. 2005. Proline-based modulation of 2,4-diacetylphloroglucinol and viable cell yields in cultures of *Pseudomonas fluorescens* wild-type and over-producing strains. Applied Microbiology and Biotechnology. (In press, on line 2-18-05 at http://www.springerlink.com/index/10.1007/s00253-005-1907-4).

Zhang, S., Schisler, D.A., Boehm, M.J., and Slininger, P.J. 2005. Carbon-to-nitrogen ratio and carbon loading of production media influence freeze-drying survival and biocontrol efficacy of *Cryptococcus nodaensis* OH 182.9. Phytopathology (accepted 2/8/05).

Screening, production and formulation technologies for biocontrol agents of Fusarium head blight of wheat

Background: The development of effective microbial selection, biomass production and biomass formulation methodologies for microorganisms capable of reducing plant diseases represents a vital step in the advance of biocontrol products that will provide attractive solutions to several vexing problems inherent with traditional plant disease control strategies. A serious impediment to bringing antagonists to the marketplace is the lack of adequate knowledge of the mass production and formulation technologies needed to produce cells that are tolerant to the stresses of large-scale cultivation, separation, processing (drying or dewatering biomass) and storage. We devise commercial practice-oriented biocontrol agent selection strategies and elucidate the genetic and metabolic mechanisms that enhance microbial stress tolerance such that high yields of viable, effective cells with long shelf-lives can be produced. In the specific case of our work in discovering and developing novel biocontrol agents active against Fusarium head blight of wheat, the new technologies developed will ultimately contribute to reducing estimated losses of at least $500 million per year attributed to this disease in the United States.

Accomplishment: Novel microbial antagonists for reducing Fusarium head blight of wheat were discovered and new liquid culture protocols and formulations were developed for producing biomass with enhanced efficacy and shelf-life. Screening microbial colonists isolated from wheat anthers for ability to utilize compounds such as tartaric acid or choline enhanced the recovery of strains capable of reducing Fusarium head blight in greenhouse and field trials. Choline utilizing strains were isolated based on the observation that choline is found in higher relative concentrations in tissues of susceptible flowering wheat heads and enhances germ tube elongation of the pathogen. Novel production techniques were developed that enabled the mass production (100 L fermentations) and processing of biomass of the yeast antagonist *Cryptococcus flavescens* (*C. nodaensis* nomen nudum) as a frozen biomass concentrate with an extended shelf-life. We discovered that formulating biomass of *C. flavescens* with the previously unreported osmoprotectant melezitose significantly enhanced the stability of freeze-dried preparations of antagonist biomass. Significant reduction of FHB symptoms were achieved when grains were treated at flowering in field tests conducted over multiple sites and years on soft red winter wheat, hard red spring wheat and durum wheat.

Impact: Technology transfer has been achieved via the issuance of two patents and one pending patent [Schisler, Khan and Boehm (2001), US Patent 6,312,940; Schisler, Khan and Boehm (2003), US Patent 6,562,337] regarding the novel biocontrol strains discovered as well as multiple peer reviewed and technology transfer publications. A Trust Fund Cooperative Research Agreement was completed with the Dakota Growers Pasta Company to conduct a field demonstration of the efficacy of several of our patented antagonists against FHB on durum wheat. Due to the demonstrated potential of our FHB biocontrol strain *C. flavescens*, the U.S. Wheat and Barley Scab Initiative funded cooperative field research that was conducted with 13 State Universities at approximately 16 field sites over two successive years. Project results led to a 2.5 year cooperative international research project with Former Soviet Union (FSU) Bioweapons Researchers that was funded through the auspices of ARS's Office of International Research Program. ARS and FSU collaborators made multiple visits to each other's laboratories, tested biocontrol strains in each others countries and published results. Invited presentations of these results by CRIS scientists at numerous national and international meetings have influenced production and formulation research of scientists in related and unrelated fields. Ultimately these results contribute to advancing biocontrol strains in general towards commercialization by solving previously intractable problems of production, formulation and stabilization of biomass that must be met before commercial product development can be reasonably anticipated.

Additional Information: Demonstration of the feasibility of reducing FHB on durum wheat using ARS-discovered antagonists was made possible in part by ARS obtaining a two year grant from the Dakota Growers Pasta Company. Additionally, ARS maintained a Specific Cooperative Agreement with The Ohio State University from 2000-2004. This SCA was funded primarily by competitive funding that was obtained by ARS from the U.S. Wheat and Barley Scab Initiative. Expertise in the initiation and maintenance of wheat field trials was provided by collaborators at The Ohio State University.

Documentation:

Schisler, D.A., Khan, N.I., Boehm, M.J., and Slininger, P.J. 2002. Greenhouse and field evaluation of biological control of Fusarium head blight on durum wheat. Plant Disease 86:1350-1356.

Schisler, D.A., Behle, R.W., Slininger, P.J., and Jackson, M.A. 2003. Production and formulation of microbial products active against plant pests. Proceedings of the II Moscow International Congress of Biotechnology:State of the art and prospects of development, P&I JSC "Maxima", Moscow, Russia. p. 263.

Khan, N.I., Schisler, D.A., Boehm, M.J., Slininger, P.J., and Bothast, R.J. 2001. Selection and evaluation of microorganisms for biocontrol of Fusarium head blight of wheat incited by *Gibberella zeae*. Plant Dis. 85:1253-1258.

Schisler, D.A., Slininger, P.J., Behle, R.W., Zhang, S., Boehm, J.J., Lipps, P.E., and Palmquist, D.E. 2003. USDA-ARS, Ohio State University cooperative research on biologically controlling Fusarium head blight 1: *in vitro* and field testing of the effect of UV protectants on FHB antagonists. Proceedings of the 2003 National Fusarium Head Blight Forum, Kinko's, Okemos, MI. pp. 105-108.

Khan, N.I., Schisler, D.A., Boehm, M.J., Lipps, P.E., and Slininger, P.J. 2004. Field testing of antagonists of Fusarium head blight incited by *Gibberella zeae*. Biological Control 29:245-255.

Discovery Area 5: **Molecular Biology and Systematics of Biocontrol Agents**

Total Genome sequencing of Pseudomonas fluorescens Pf-5

Background: *Pseudomonas fluorescens* Pf-5 inhabits the root surfaces (rhizosphere) of many plants and functions as a biological control agent, suppressing a number of plant diseases caused by soilborne plant pathogens. Pf-5 produces a large spectrum of secondary metabolites, including several antibiotics. The selection of *P. fluorescens* Pf-5 as the subject for genomic sequencing was based upon the importance of Pf-5 as a biological control organism, its rhizosphere competence, the large spectrum of antibiotics and other secondary metabolites that it produces, and its status as model environmental strain for studies of gene regulation. Strain Pf-5 was described early in the history of biological control research (Howell and Stipanovic 1979, 1980), and it was the first biological control agent for which the chemical basis of disease suppression was known.

Accomplishment: The genome of the biological control agent *Pseudomonas fluorescens* Pf-5 was completely sequenced, and this is the first biological control agent for plant disease whose sequence is known. The genome was sequenced at the Institute for Genomics Research, under the direction of Ian Paulsen, in collaboration with ARS scientists and researchers at the University of Arizona and Rutgers University. The

genomic sequence of Pf-5 highlights several important characteristics of the biological control agent, including its production of multiple antibiotics toxic to plant pathogens, its utilization of plant-produced nutrients, its capacity to utilize siderophores produced by a broad range of soil microorganisms, and the lack of genes required for pathogenicity. Sequence data will be used to develop basic knowledge of biological control, with the purpose of improving the consistent efficacy of biological control in agriculture.

Impact: Sequence data is posted on a publicly accessible website that is used worldwide for access to genomic sequence data. The availability of a genomic sequence for a biological control agent will now allow scientists to employ a range of post-genomics technologies to enhance knowledge of biological control. The genome of Pf-5 can now be compared to the genome of related bacteria such as *Pseudomonas aeruginosa* and *Pseudomonas syringae*. Comparative genomics will advance knowledge of traits involved specifically in pathogenesis, as well as those involved in ecological fitness on plant surfaces or in soil.

Additional Information: Outside funding from the USDA-CSREES Microbial Genome Sequencing Program was essential for this accomplishment, as was collaboration with The Institute for Genomic Research, Rutgers University, and the University of Arizona was key.

Improving Pseudomonas fluorescens strains for biocontrol of soilborne pathogens of wheat

Background: Root diseases caused by soilborne fungal pathogens are major production limiting factors in cereal-based cropping systems, particularly when several consecutive crops are planted and when crops are direct-seeded into their own stubble (no-till). Wheat and barley lack resistance to many of the most common root pathogens, and populations of antagonistic rhizobacteria provide the first line of defense. These biological control bacteria suppress pathogens by producing phenazines and phloroglucinol antibiotics on the roots, but disease suppression can fail due to poor root colonization and inadequate antibiotic production. The goal of this ARS research is to develop bacterial biological control agents that consistently and effectively control root diseases caused by major cereal root fungal pathogens.

Accomplishment: Strains of *Pseudomonas fluorescens* with superior root colonization ability and enhanced antibiotic production were developed by using classical and molecular microbiological approaches. Genomic fingerprinting techniques identified 18 unique BOX-PCR genotypes (A-Q & T) among 2,4-diacetylphloroglucinol (DAPG)-producing fluorescent *Pseudomonas* spp. D-genotype strains, exemplified by *P. fluorescens* Q8r1-96, showed a unique ability to aggressively colonize and persist on the roots of wheat and are responsible for take-all decline in Washington State wheat fields. Introduction of the biosynthetic locus for the antibiotic phenazine-1-carboxylic acid stably into strain Q8r1-96 resulted in transgenic strains that retained DAPG production, constitutively produce phenazine antibiotics, retained superior root colonizing ability, and were effective at lower inoculum doses and against a broader spectrum of fungal

pathogens than strain Q8r1-96 or the phenazine gene donor strain. Despite having been known for over a century, the reactions involved in phenazine synthesis have remained obscure. ARS scientists and two groups of collaborators identified key genes, intermediates and novel steps in the biosynthetic pathway, opening new opportunities to improve the performance of phenazine-producing biocontrol agents. Genes unique to D-genotype strains as a first step to defining molecular determinants of their unique affinity for wheat roots and superior rhizosphere competence were also identified.

Impact: These studies show that entire pathways for the synthesis of antifungal metabolites can be combined to generate superior biological control agents. Our strains are the basis of the longest-running risk assessment analysis of the effects of recombinant biocontrol bacteria in the United States. These strains have had no impact on non-target indigenous microbial populations in the rhizosphere of wheat, which may help to alleviate concerns about the introduction of recombinant strains into the environment. The strains also are a valuable research tool to identify and compensate for local factors limiting to the performance of introduced biocontrol agents. Although the antibiotic properties and pharmacological potential of phenazine antibiotics have been recognized for over a century, our research is the first in over 30 years to provide new insight into key reactions involved in their synthesis. The results will be used to target phenazine production more specifically to particular plant pathogens. Some phenazines produced by animal and human pathogens are virulence factors, and others have anticancer activity; our results also therefore are broadly applicable in drug design and disease treatment.

Additional Information: Competitive grants supported research that contributed to this accomplishment:

O. A. Vogel. Wheat Research Fund.

Documentation:

Mavrodi, D. V., Bonsall, R. F., Delaney, S. M., Soule, M. J., Phillips, G., Thomashow, L. S. 2001. Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. Journal of Bacteriology. V. 183 p. 6454-6465.

Mavrodi, O. V., McSpadden Gardener, B. B., Mavrodi, D. V., Bonsall, R. F., Weller, D. M. Thomashow, L. S. 2001. Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* species. Phytopathology. V. 91. p 35-43.

McDonald, M., Mavrodi, D. V., Thomashow, L. S., Floss, H. G. 2001. Phenazine biosynthesis in *Pseudomonas fluorescens*: branchpoint from the primary shikimate biosynthetic pathway and role of phenazine-1,6-dicarboxylic acid. Journal of the American Chemical Society. V. 123 p.9459-9460.

Blankenfeldt, W., Kuzin, A., Skarina, T., Korniyenko, Y., Tong, L., Bayer, P., Janning, P., Thomashow, L. S., Mavrodi, D. V. 2004. Structure and function of the phenazine

biosynthetic protein PhzF from *Psesudomonas fluorescens.* Proceedings of the National Academy of Sciences of the USA. V. 101 p.16431-16436.

Huang, Z., Bonsall, R. F., Mavrodi, D. V., Weller, D. M., Thomashow, L. S. 2004. Transformation of *Pseudomonas fluorescens* with genes for biosynthesis of phenazine-1-carboxylic acid improves biocontrol of Rhizoctonia root rot and in situ antibiotic production. Federation of European Microbiology Societies Microbiology Ecology. V. 49 p. 243-251.

Molecular interactions between Pseudomonas fluorescens and wheat roots

Background: Biological control offers a sustainable means of controlling fungal soilborne pathogens of small grain cereals, particularly in the face of emerging root diseases and in the absence of effective genetic resistance in the host. Biocontrol isolates of the rhizobacterium *Pseudomonas fluorescens* exhibit preferences for specific crop species, and differ in their abilities to persist in the rhizosphere. *P. fluorescens* genes that mediate rhizosphere competence have been isolated, but contributing host-derived genes and mechanisms are unknown. Furthermore, the action of *P. fluorescens*-derived antifungal metabolites such as 2,4-diacetylphloroglucinol (DAPG) on host molecular and cellular processes remain poorly characterized.

Accomplishment: Twenty-eight Pacific Northwest cultivars of hexaploid wheat were evaluated for their ability to undergo and sustain root colonization by two *P. fluorescens* isolates that vary in rhizosphere competence. Five cultivars supported higher rhizosphere populations of the aggressive root colonizing isolate Q8r1-96 (BOX-PCR genotype D) as compared to the less aggressive isolate Q2-87 (BOX-PCR genotype B), whereas other cultivars supported either high or low population sizes of both isolates. The phenotypes of the two *P. fluorescens* strains are virtually identical, and differ only in their molecular fingerprint. The findings were used to select "model" cultivars for subsequent studies of gene expression and DAPG accumulation. A microarray-based approach was used to determine if defense gene homologs of the model plant *Arabidopsis* are induced or repressed in wheat roots during colonization by *P. fluorescens*. Genes encoding a regulator of jasmonate signaling and proteins involved in oxidative stress were induced in wheat roots upon bacterial colonization. Changes in expression of the genes were authenticated using real-time PCR.

Impact: The findings demonstrate that host factors contribute to the rhizosphere competence of introduced rhizobacteria, and lay the foundation for genetic and molecular approaches to dissecting the relevant pathways and genes in wheat. This research also provides the first insight into two root-localized defense pathways triggered by aggressive biocontrol isolates, and suggests other mechanisms by which disease suppressiveness by biocontrol bacteria are achieved in wheat. The pathways will be a starting point for enhancing the efficacy of rhizobacteria in an important crop plant.

Documentation:

Okubara, P.A., Kornoely, J.P., Landa, B.B. 2004. Rhizosphere colonization of hexaploid wheat by *Pseudomonas fluorescens* strains Q8r1-96 and Q2-87 is cultivar-variable and associated with changes in gross root morphology. Biological Control. V. 30 p. 392-403.

Systematics of Biocontrol Fungi: Trichoderma and Hypocrea

Background: Cacao is a tropical crop grown in almost all tropical regions. Its major economic effect in the United States is in the secondary products that are used in the preparation of chocolate and chocolate products. These include, but are not limited to, milk, wheat, nuts and fruit. Cacao is beset with two major diseases in tropical America and one major disease in West and Central Africa. These are witches' broom disease and frosty pod rot in America and black pod caused by *Phytophthora megakarya* in Africa. Chemical controls for these diseases are of limited effectiveness and are only economically useful when the price of cocoa is high. The use of chemical controls removes the crop from the growing organic market while, at the same time, degrading the environment. Biological control alternatives are being sought that can either protect the crop (through induced resistance or a direct means) or can reduce the amount of inoculum.

Accomplishment: This work has developed the ability to identify quickly unknown isolates of *Trichoderma* and to recognize undescribed species. A phylogeny of the genus *Trichoderma* has been developed using DNA sequencing and phenotype characterization. Partial monographs for groups of species of *Trichoderma* have been published. Isolates of *Trichoderma* have been received for identification from plant pathologists all around the, primarily, tropical world. Five have been shown to be effective in crop management, viz. *T. asperellum* against *Phytophthora megakarya* in cacao on Cameroon, the new species *T. ovalisporum* protecting against frosty pod rot in Costa Rica and two new species effective as protectants against frosty pod rot in Ecuador.

Impact: The newly described species *T. stromaticum* is the active agent in the patented product TRICHOVAB, effective in reducing inoculum potential of the Witches' Broom pathogen in Bahia, Brazil. Prior to the development of a multigene phylogeny for *Trichoderma* species names were not accurately applied with the effect that communication about *Trichoderma* species could not be effective. For example, our recent work on systematics of the *T. koningii* species complex species demonstrated that many reports in the literature of *T. koningii* used in biological control are based on misidentifications. The biological control species *T. ovalisporum* and one of the new species from Ecuador were initially identified as *T. koningii*.

Additional Information: Outside collaborations were significant and essential. Outside collaborators included USDA-ACSL lab (now SPCL lab), MasterFoods, USA; INIAP in Ecuador, IRAD in Cameroon, CABI-Bioscience in UK, IPARC in UK and CATIE in Costa Rica. Outside collaborators provided isolates and undertook field trials with identified strains.

Documentation:

Holmes, K., Schroers, H.-J., Thomas, S. E., Evans, H. C., Samuels, G.J.. 2004. Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. Mycological Progress 3: 199-210.

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Samuels, G. J., Dodd, S. L., Gams, W., Castlebury, L. A., Petrini, O. 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia 94: 146-170.

Samuels, G. J., Pardo-Schultheiss, R., Hebbar, P., Lumsden, R. D., Bastos, C. N., Costa, J. C., Bezerra, J. L. 2000. *Trichoderma stromaticum*, sp. nov., a parasite of the cacao witches broom pathogen. Mycological Research 104: 760-764

Discovery Area 6: Biocontrol of Insect Vectors of Pierce's Disease

Novel viral pathogen of sharpshooter

Background: Naturally occurring pathogens greatly reduce insect populations but may go unnoticed. Discovery and characterization of new insect pathogens will aid management programs; combining these discoveries with the elucidation of insect genes that regulate critical developmental stages may lead to more efficient plant/insect pest management strategies.

Accomplishment: New sharpshooter viruses have been discovered and are being characterized. A provisional patent application has been filed. Approximately 20,000 EST have been produced and published from the GWSS the vector of Xylella caused diseases. An additional 20 full length genes and proteins have been released into the NCBI public database

Impact: The new discovery of insect viruses specific to sharpshooters has opened a new area of research and focused efforts to integrate these biological control agents into existing IPM programs. Entomopathogenic viruses are likely to reduce sharpshooter populations and limit spread of the disease.

Additional Information: Development of genetic markers from grapes for marker-assisted selection in has been a collaborative effort with the Florida A&M University. ARS provided the expertise to conduct EST sequencing and marker identification, and the Florida A&M University's responsibility is to screen and characterize the markers within his grape breeding program.

Documentation:

Hunter, W.B., Lapointe, S.L., Sinisterra, X.H., Achor, D.S., Funk, C.J. 2003. Iridovirus in *Diaprepes* Root Weevils (Coleoptera: Curculionidae: *Diaprepes abbreviatus*). Journal of Insect Science 3:9.

Hunter, W.B., P.M. Dang, M.G. Bausher, J. X. Chaparro, W. McKendree, R.G. Shatters, Jr., C.L. McKenzie and X.H. Sinisterra. 2003. Aphid Biology: Expressed Genes from Alate *Toxoptera citricida* (Kirkaldy), Brown Citrus Aphid (Hemiptera: Aphididae). 7pp. Journal of Insect Science 3:23.

Shatters, RG, Jr., Bausher, MG, Hunter, WB, Chaparro, JX, Dang PM, Niedz, RP, Mayer, RT, McCollum, TG, and XH. Sinisterra. 2003. Putative protease inhibitor gene discovery and transcript profiling during fruit development and leaf damage in grapefruit (*Citrus paradise* Macf.). GENE 326: 77-86.

Hunter, W.B., C.P. Patte, X.H. Sinisterra, D.S. Achor, C.J. Funk and J.E. Polston. 2001. Discovering new insect viruses: Whitefly iridovirus (Homoptera: Aleyrodidae: *Bemisia tabaci*). Journal of Invertebrate Pathology 78: 220-225).

Funk, C.J., W.B. Hunter and D. S. Achor. 2001. Replication of Insect Iridescent Virus 6 in a Whitefly Cell Line. Journal of Invertebrate Pathology, 77(2): 144-146.

Cultural Control Component

Background: Cultural methods for managing plant diseases are often inadequately exploited. Improved cultural practices offer a general strategy for disease management with minimal environmental impact. Knowledge of how tillage practices, crop rotations, water management, and other practices affect pathogen survival, disease initiation, and disease spread is needed to obtain the maximum benefits.

Discovery Area 1: Natural Products and Soil Amendments Reduce Pathogen and/or Disease Development

Natural products and soil amendments are inadequately utilized sources of disease reduction technologies. ARS scientists discovered a variety of products and amendments with application in reducing plant disease in the following areas.

Comment [A1]: Schisler comment

Undiagnosed disorders in pecan orchards have become increasingly frequent and severe in recent years. These maladies have also evolved into a replant disease issue affecting orchard establishment efforts. The cause of the disorder has over the decades been attributed to any one of a host of biotic and abiotic agents.

Comment [A2]: Wood

Nematodes cause nearly ten billion dollars in crop losses per year in the United States and nearly $100 billion globally. For some crops, such as soybeans, nematodes are the most important pest or pathogen. However, many of the conventional nematicides that were used to control plant-parasitic nematodes have been shown to contribute to groundwater contamination and to be hazardous to the health of humans and animals, and have therefore been banned or restricted in use. It is particularly notable that the restrictions on methyl bromide application will remove the most widely used nematicide from agricultural use. Consequently, new, effective and environmentally safe tools for managing nematodes are urgently needed. ARS scientists are developing nematode management systems based on strategies that include identifying natural products that adversely affect nematodes and applying soil amendments to reduce nematode crop losses.

Comment [A3]: Meyer

Greenhouse floricultural crops represent one of the largest commodities of American agriculture. Improvements in greenhouse crop culture are required to enable American producers to remain competitive in world markets. A joint research project, between the ARS and the University of Toledo (UT), is focused on hydroponic and soilless crop culture addressing the most important greenhouse stress management, disease, and insect problems as determined through interaction with the commercial greenhouse production industry. Proper management of crops, diseases, and insect problems will enhance profitability and competitiveness of American growers.

Comment [A4]: Locke

Pecan disorders traced to nickel deficiency

Accomplishment: It was discovered that the cause of a growth malady (mouse-ear) and a replant disease, or disorder, that has become increasingly common in commercial pecan orchards, is a nickel (Ni) deficiency. It was also found that Ni deficiency is largely due to excessive application of standard fertilizer nutrient elements to orchards (e.g., Ca, Mg, Fe, Mn, Cu, and Zn). Thus, it was found that there has been excessive long-term fertilization of several mineral elements, especially that of zinc. A Ni management strategy was developed that enables farmers to protect against economic losses associated with Ni deficiency

Comment [A5]: Wood

Impact: Nickel deficiency was discovered for the first time to actually exist in real-world agriculture, thus raising the possibility that many other agricultural crops suffering from replant problems or undiagnosed maladies may in fact be associated with a Ni deficiency being induced by fertility practices or root pathogens. The work has affirmed that Ni is indeed an essential plant nutrient, has drawn attention to the fact that Ni nutrition of plants merits monitoring, has promoted marketing of Ni fertilizers, has promoted state regulatory bodies to authorize Ni usage in U.S. agriculture, and has produced a Provisional Pending Patent on Ni usage.

Deleted: ¶

Additional Information: Outside funding from The Georgia Agricultural Commodity Commission for Pecans provided monetary support for this project research.

Comment [A6]: Wood

Documentation:

Wood, B. W., Reilly, C. C., Nyczepir, A. P. 2004. Mouse-ear of pecan: A nickel deficiency. HortScience 39(6):1238-1242.

Wood, B.W., Reilly, C.C., Nyczepir, A.P. 2004. Mouse-ear of pecan: I. symptomology and occurrence. HortScience. 39(1):87-94.

Wood, B.W., Reilly, C.C., Nyczepir, A.P. 2003. Cu and Ni deficiency symptoms and mouse-ear in pecan. HortScience. 38(5):726.

Alkaline-stabilized biosolid discovered to be effective against SCN and RKN as a soil amendment

Accomplishment: The incorporation of organic amendments into agricultural soils offers potential benefits for both nematode control and improvement of soil fertility. Therefore, ARS scientists and collaborators evaluated the effect of an alkaline-stabilized biosolid soil amendment on SCN and RKN and on soil chemical properties, and investigated factors that may influence the efficacy of the amendment for suppressing plant-parasitic nematodes. The amendment was suppressive against both nematode species, and nematode suppression was positively correlated with pH, calcium, sulfur and ammonia concentrations in the soil solution. Replacement studies demonstrated that an increase in soil pH was the soil chemical characteristic most closely related to nematode suppression, with ammonia production being secondary. Studies also demonstrated that alkaline stabilization of biosolids was necessary to achieve nematode suppression, that

microbes associated with the amendment appeared not to be responsible for the nematode suppressive activity, that alkaline-stabilized biosolids from different processing facilities all provided nematode suppression, and that the ability of this amendment to suppress RKN on tomato was influenced by soil type. Additionally, several temperate and tropical cover crops are being evaluated for nematode suppression in vegetable, grape and soybean production systems.

Comment [A7]: Meyer

Impact: The research is important because it provided an understanding of the mode of nematode suppression of an alkaline-stabilized biosolid amendment, and of factors that influence the efficacy of the amendment. The ability of this amendment to suppress nematodes in a growers' field will depend on soil type. The work also indicated that alkaline-stabilized biosolids from several geographic locations were effective in suppressing plant-parasitic nematodes. This will allow for the widespread use and marketing of this product as a consistent and reliable nematode management option. Research evaluating cover crops for plant-parasitic nematode suppression have identified the toxicity of specific plant-derived compounds against nematodes potentially leading to the more reliable use of these plants as nematode management tools.

Comment [A8]: Meyer

Additional information: ARS scientists collaborated with researchers at the following locations: 1) N-Viro International, Toledo, Ohio; University of Florida, Homestead, Florida; University of Michigan, East Lansing, Michigan; University of Iowa, Ames, Iowa; North Carolina State University, Raleigh, North Carolina; Ohio State University, Columbus, Ohio; and University of Manitoba, Winnipeg, MB, for alkaline-stabilized biosolids research; and 2) Pennsylvania State University, Bigglerville, Pennsylvania; University of Maryland, College Park, Maryland; and University of Florida, Homestead, Florida for the identification and evaluation of the chemical components of cover crops toxic to nematodes.

Comment [A9]: Meyer

Documentation:

Zasada, I.A. and M. Tenuta. 2004. Chemical-mediated toxicity of N-Viro Soil to *Heterodera glycines* and *Meloidogyne incognita*. Journal of Nematology 36:297-302,

Chitwood, D.J. 2002. Phytochemical based strategies for nematode control. Annual Review of Phytopathology 40:221-249.

Commercial greenhouse protocols studied

Accomplishment: ARS scientists developed an understanding of the commercial greenhouse industry through personal visitations to build stakeholder rapport and to identify the priority, researchable problems facing growers. Initial research studies have included evaluation of water-holding polymers in growing media, critical evaluation of specific nutrients (N, P, Fe and B), the uptake and fate of Si, and the effect of fertilizer anion species on growth, development, and disease expression. Initial findings have been reported back to the grower community through informal grower-based meetings and poster presentations at national scientific meetings. Major equipment purchases and

installation has outfitted this startup site with the facilities necessary to conduct plant growth research to address the needs identified in the commercial greenhouse industry. Additional support staff has been hired and outside researchers, both at the University of Toledo (UT) and other universities, have been identified to support specific aspects of the research through Specific Cooperative Agreements. A regional Liaison Committee consisting of greenhouse producers and farm market operators, Cooperative Extension personnel, and allied stakeholders has been formed to provide assistance in understanding priority research needs, give knowledgeable feedback, provide a mechanism for technology transfer, and give credibility to the research mission.

Comment [A10]: Locke

Impact: The infrastructure for this new project and rapport with the immediate stakeholders has been established, significant research partnerships have been formalized through Specific Cooperative Agreements and research productivity is occurring with initial technology transfer activities established through both scientific channels and directly to stakeholders, all of which will contribute to the goal of improving greenhouse crop production. These improvements in production technology are being transferred directly to the stakeholders and will lead to enhanced profitability and competitiveness.

Comment [A11]: Locke

Additional information: Specific Cooperative Agreements (SCAs) have been established during this past year with non-ARS scientists located at the University of Toledo (UT), North Carolina State University (NCSU), University of Florida (UF), and Ohio State University (OSU). The SCA with UT is focusing on molecular mechanisms of plant response to abiotic and biotic stress that will lead to methods of rapid, non-destructive stress determination in plants in order to address the problem during the production cycle to yield high value crops. The SCA with NCSU is directed to look at nutritional and pH relationships during production of geranium, the most important bedding plant species in American floriculture. The SCA at UF is collaborating efforts to evaluate the uptake and potential role of silicon in bedding plants for crop improvement and disease resistance. The SCA at OSU is focused on determining the role of the environment in nutrient uptake and partitioning in petunia.

Comment [A12]: Locke

This greenhouse-based project was initially funded in FY 2001, but since it was directed to be established at a previously non-ARS location, the project had to be completely established with no staff, laboratory, equipment, or even a greenhouse. The two ARS scientists were recruited and hired in FY 2002-2003 and support hires were made in FY 2003-2004.

Comment [A13]: Locke

Documentation:

Bowers, J.H. and Locke, J.C. 2004. Effect of formulated plant extracts and oils on population density of *Phytophthora nicotiannae* in soil and control of Phytophthora blight in the greenhouse. Plant Dis. 88:11-16.

Frantz, J.M., Pitchay, D.S., Locke, J.C., Horst, L.E. and Krause, C.R. 2005. Silicon is deposited in leaves of New Guinea Impatiens. Online. Plant Health Progress doi:10.1094/PHP-2005-0217-01-RS.

Locke, J.C., Pitchay, D.S. and Frantz, J.M. 2004. Effect of nitrogen, potassium and silicon nutrition on disease susceptibility of various ornamental crop species. Phytopathology 94:S62. (Abstr.)

Frantz, J.M., Pitchay, D.S., Locke, J.C., Horst, L.E. and Krause, C.R. 2004. Evaluating silica uptake in bedding plants. HortScience 39(4):776. (Abstr.)

Pitchay, D.S., Frantz, J.M., Locke, J.C. and Krause, C.R. 2004. Managing nitrogen, potassium, and boron in bedding plants: Is more better? HortScience 39(4):856. (Abstr.)

Discovery Area 2: Chemical Treatments Impact Pathogen and/or Disease Development

Chemical alternatives to methyl bromide and new uses for proven chemical control compounds are highlighted in this area of ARS research. Several projects focus on determining improved management strategies using chemicals for important diseases of small fruits, nuts and deciduous tree crops. Phosphonate treatment for reducing Perennial Phytophthora Canker on almonds is reported as is the use of chloropicrin to replace methyl bromide in combating Prunus Replant Disease (PRD) on almond and peach.

Comment [A14]: Kluepfel, Schisler

ARS research to determine effective fungicides for management of Sclerotinia in sunflower and dry edible bean was conducted as part of The National Sclerotinia Initiative. The Initiative is a coordinated research effort aimed at reducing the economic threat of Sclerotinia in sunflower, soybean, canola, dry edible beans, chickpeas, lentils, and dry peas. More details regarding this consortium are presented in NP303 component IV report.

Comment [A15]: Chandler

Comment [A16]: Gary, this should be true but please check final drafts of our reports to confirm this statement is true.

Pecan scab disease and shuck decline are key economically important diseases affecting the U.S. pecan industry. Effective and economical control has been a major challenge for the southeastern sector of the industry. This is because of the difficulty of preventing nut yield losses, damage to foliage, impact on alternate bearing, and the economic loss associated with several cover sprays. Additionally, yield and tree loss to mistletoe is a severe problem in certain locations.

Comment [A17]: Wood

Due to increasingly stricter water-use and run-off regulations, greenhouse and nursery operations capture water run-off that inadvertently contains pathogens. Irrigation water pumped from the catchment ponds redistributes pathogens onto the crops. Few disinfestants are economically suitable for killing pathogens when pumping a high gallonage of water, as is typical of greenhouse and nursery production. Chlorine gas, the most prevalently used disinfestant, may be restricted by regulations in the future. Chlorine dioxide is a disinfestant that is commercially used by many industries including treatment of municipal drinking water but had not been evaluated for use in ornamental plant production systems. In addition to being effective, a disinfestant must pose minimal potential to cause damage on the hundreds of plant genera grown by ornamental plant producers.

Comment [A18]: Spiers

Perennial Phytophthora canker (PPC) reduced using phosphonate

Accomplishment: ARS scientists determined the efficacy and persistence of phosphonate treatments to prevent PPC of almond in numerous replicated orchard trials. The research determined optimized methods and timing for phosphonate treatments in the almond industry.

Comment [A19]: Kluepfel

Impact: Perennial *Phytophthora* canker has caused severe losses in many almond orchards of the San Joaquin Valley, and the identification and optimization of effective phosphonate treatments has provided valuable control measures to the almond industry.

Comment [A20]: Kluepfel

Additional information: The ARS scientist collaborated in team research funded by USDA CSREES that targeted evaluations of methyl bromide alternatives for strawberry and deciduous fruit and nut crop industries.

Documentation:

Browne, G.T., Viveros, M.A. 2005. Effects of phosphonate and mefenoxam treatments on development of perennial cankers caused by two *Phytophthora* spp. on almond. Plant Disease 89:241-249.

Browne, G.T., Wilcox, W.F., Latore. 2005. Phytophthora crown and root rot. In: Compendium of Grape Diseases. The American Phytopathological Society, St. Paul, Minnesota.

Fumigant alternatives to methyl bromide developed to combat Prunus Replant Disease (PRD)

Accomplishment: Prior to determining the PRD causative agent(s) ARS scientists developed an integrated management strategy for PRD on almond and peach that incorporates the use of several fumigant alternatives to methyl bromide that are applied to individual tree planting site locations. The most effective was chloropicrin, which prevented PRD at application rates equivalent to standard methyl bromide applications. The efficacy of the alternative chloropicrin treatments has significant implications for the fruit and nut industries. In addition, ARS scientists showed that short-term pre-plant fallowing and cover crop rotations are also effective in the prevention of PRD. Finally, they have provided evidence that there is cross specificity between peach and grape PRD which will have significant implications both the Almond and Grape industries.

Comment [A21]: Kluepfel

Impact: Prunus Replant Disease is a significant limiting factor to Almond Production in California which is more than a billion dollar industry in the state. ARS scientists developed several cost effective control strategies for this disorder that have been accepted by the industry. In addition these new control measures no longer require the use of methyl bromide which was the industry standard as recent as a 3-4 years ago. In addition, several control strategies have been developed which are independent of chemical fumigation.

Comment [A22]: Kluepfel

Additional information: Research support from the Almond Board of California augmented USDA-ARS base funded research on biology and management of Phytophthora diseases on Almond and Prunus Replant Disease on Almond.

Documentation:

Westphal, A., Browne, G.T., and Schneider, S. 2002. Evidence for biological nature of the grape replant problem in California. Plant and Soil 242:197-203.

Browne, G.T., DeTar, W.R., Sanden, B.L., Phene, C.J. 2002. Comparison of drip and sprinkler irrigation systems for applying metam sodium and managing stem rot of potato. Plant Disease 86:1211-1218.

Fungicides and information transfer enabled in the effort to reduce Sclerotinia diseases

Accomplishment: Numerous accomplishments have occurred as a result of the efforts of the research consortium that comprises the National Sclerotinia Initiative. To date, research and technology transfer activities conducted through the Sclerotinia Initiative have resulted in numerous achievements in the areas of genetics and breeding, disease epidemiology, and crop management practices. Two accomplishments most appropriately reported here include: 1) Establishment of a comprehensive Sclerotinia Initiative website to serve the needs of the agricultural community and to provide educational information to the general public and 2) Determination of effective fungicides for management of Sclerotinia in sunflower and dry edible bean. More details about the National Sclerotinia Initiative are presented under the component IV report.

Comment [A23]: Chandler. NOTE, this is the extent to which the accomplishment and impact are described making assignment of impact criteria difficult

Impact: The research conducted under this broad project has had significant impact on improving the current and future management strategies for this important disease. The collective annual losses for the crops participating in the Initiative have been as high as $252 million. Research and technology transfer activities conducted by the Initiative have already begun to have a positive impact by identifying methods to accurately predict disease incidence and thus provide growers with optimal integrated management tools to address disease infestations and providing new chemical fungicide management tools to reduce annual field losses.

Comment [A24]: Chandler

Advances in controlling pecan scab and shuck decline

Accomplishment: The ability to control pecan scab disease was enhanced by the discovery that residual activity from scab fungicides was much less than commonly accepted, thus leading to spray interval modifications in spray strategies. It was also found that early season scab sprays were also protecting from damage caused by shuck decline disease appearing in late summer; thus establishing a linkage between the two diseases and their control strategies. Additionally, a method utilizing chemical treatment

of mistletoe clusters 2-3 weeks prior to budbreak was developed that enabled safe eradication of mistletoe from tree canopies.

Impact: Research efforts produced scab disease and shuck decline disease control strategies that are being used by farmers to reduce associated yield losses. The work has also targeted poor spray coverage of tree canopies as a primary contributing factor to the inability of farmers to control scab disease in wet years, thus acting to reduce losses by reinforcing the need for farmers to ensure good canopy coverage by protective sprays in wet years. This knowledge is leading to less economic loss to pecan scab and the production of a higher quality nut product from the southeastern United States. The development of a method of safely eradicating mistletoe from trees enables avoidance of tree and yield losses due to parasitism by this pest.

Comment [A25]: Wood

Additional information: Collaborative research was also conducted with University of Georgia to develop new spray technologies for improved control of a disease on peach. An air assisted rotary atomizer spray technology was shown to be superior to air-blast sprayers for pest control in peach orchards, while simultaneously reducing pesticide drift by 90% and using less water per acre and requiring less time for spraying. The technology is available for commercial usage and is in the early stages of being adopted by farmers.

Comment [A26]: Wood

Documentation:

Wood, B.W., Reilly, C.C. 2004. Control of mistletoe in pecan trees. HortScience. 39(1):110-114.

Reilly C. C., Taylor K. C., Hotchkiss M. W. 2004. A comparison of airblast and air assisted rotary atomizer spray technologies in peach production. HortTechnology. 14(4): 555-559

Chlorine dioxide discovered to kill pathogens in irrigation water

Accomplishment: ARS scientists demonstrated that low rates of chlorine dioxide would kill the common spore types of *Fusarium oxysporum* and *Thielaviopsis basicola* but rates need to be adjusted for variants in water properties (pH, water hardness, and nutrient leachates) that vary considerably between regional and national water sources. Phyto-tolerance of ornamental bedding and woody plant species to two disinfectants, with more tolerance to high rates of chlorine dioxide than to hydrogen dioxide.

Comment [A27]: Spiers

Impact: This research establishes that chlorine dioxide is commercially suitable for disinfesting irrigation water. The rate guidelines that ARS scientists defined give private industry the basic tools needed to develop computerized mixing and injection systems that will regulate chlorine dioxide levels. Furthermore, the research demonstrated that plants were not sensitive to chlorine dioxide at the rates needed to disinfest irrigation water.

Comment [A28]: Spiers

Additional information: The research was done in collaboration with Washington State University (WSU). WSU scientists had done research previously with chlorine dioxide for treating ornamental bulb crops in large commercial dump tanks and contributed significantly to developing experimental methods and design.

Comment [A29]: Spiers

Documentation:

Copes, W. E., Chastagner, G. A., and Hummel, R. L. 2001. Influence of select inorganic ions and pH on fungicidal activity of chlorine dioxide in water. Southern Nursery Assoc. Res. Conf. Proc. 46:282-284.

Copes, W. E., Chastagner, G. A., and Hummel, R. L. 2002. Toxicity responses of herbaceous and woody ornamental crops to chlorine and hydrogen dioxides. Southern Nursery Assoc. Res. Conf. Proc. 47:215-218.

Copes, W. E., Chastagner, G. A., and Hummel, R. L. 2003. Toxicity responses of herbaceous and woody ornamental crops to chlorine and hydrogen dioxides. Online. Plant Health Progress dol:10.1094/PHP-2003-0311-01-RS.

Copes, W. E., Chastagner, G. A., and Hummel, R. L. 2004. Activity of chlorine dioxide in solution of ions and pH against *Thielaviopsis basicola* and *Fusarium oxysporum.* Plant Dis. 88:188-194.

Discovery Area 3: Heat Therapy to Minimize Pathogen Contamination of Plant Materials.

Commercial varieties of sugarcane grown in the United States have a narrow genetic base. Importation of genetic resources from centers of origin and foreign breeding programs is needed to introduce desirable horticultural and pest resistant traits into new commercial lines. Germplasm from offshore origins commonly carry exotic pests, particularly viruses, which pose a threat to U.S. sugarcane production. Without methods to eliminate these pests from the imported plant propagative materials, much of the desired genetic resources will not be released from quarantine and available for use in breeding programs. *In vitro* therapy offers an avenue for eliminating virus pathogens from the infected plant material.

Comment [A30]: Hurtt

Heat therapy initiated to allow access to Saccharum spp. breeding material

Accomplishment: Various tissue culture methods and media for establishing and maintaining *Saccharum* spp. *in vitro* were evaluated since species other than *S. officinarum* (sugarcane) come into the quarantine program. A group of *Saccahrum* plants with different viruses of interest were collected. *In vitro* plantlets were established from these greenhouse plants after selecting an optimal protocol for growing *Saccahrum in vitro*. Meristem tip culture alone and in combination with heat therapy was evaluated to determine survival rates of the treated progeny. These survivors will be evaluated for efficacy in eliminating the target pathogen. This work has led to the establishment of an

in vitro system for tissue culture of *Saccharum* spp. and methods to heat treat and meristem tip culture them.

Comment [A31]: Hurtt

Impact: The development of a tissue culture program will also improve the overall operating efficiency of the sugarcane quarantine program in several ways. For example, it will provide a backup system for maintaining germplasm while it is in quarantine and undergoing testing for pathogens. It will also provide a safe haven for germplasm while plants of the cultivar/species are growing in the greenhouse and at risk of accidental cross-infection from other cultivars/species simultaneously undergoing indexing, but with different health statuses. Perhaps more importantly, the tissue culture program will allow us to move ahead in finding efficient *in vitro* methods for the elimination of viruses from imported sugarcane varieties and *Saccharum* species. These genetic resources are needed in sugarcane breeding programs and in genetic resources conservation programs. The latter are made available to scientists worldwide. Without the *in vitro* clean-up program, much of the international exchange of germplasm will not be possible.

Comment [A32]: Hurtt

Additional information: The tornado that struck the BARC-West, Beltsville, Maryland campus in September 2001, destroyed the sugarcane quarantine greenhouses. Only a small amount of greenhouse space was available in Bldg. 580 to house this program until the late fall of 2004. This reduced the amount of *Saccharum* germplasm that could be imported and indexed.

Discovery Area 4: Crop Rotations Affect Pathogen and/or Disease Development

A wide variety of ARS research projects investigated how crop rotations could be more effectively utilized to mitigate plant diseases. In the case of the reniform nematode, which has increased during the past few years in cotton fields from Georgia to Texas, newer cotton varieties have not significantly enhanced yield due likely to this nematode. Until cotton varieties with resistance to reniform nematode are commercially available, growers must turn to alternate strategies to manage the population of this pathogen and minimize yield loss. Cultural practices that can be altered to improve control of the nematode are being identified.

Comment [A33]: Young

Soilborne diseases of nursery and other horticultural crops cause serious losses to growers in the United States and throughout the world. Control or management strategies are limited at present, with growers relying heavily on chemical agents to eliminate or suppress these diseases. Crop rotations are relatively unexplored in this area. *Tomato ringspot virus* (ToRSV) is an important disease of red raspberry that is vectored by the dagger nematode, *Xiphinema americanum.* Field trials were conducted to evaluate crop rotation for the management of ToRSV as an alternative to soil fumigation

Comment [A34]: Linderman

Comment [A35]: Linderman

The major nematode pests in the three leading peach producing states (California, South Carolina, and Georgia) include ring (*Mesocriconema xenoplax*), root-knot (*Meloidogyne* spp.), and (or) root-lesion (*Pratylenchus vulnus*) nematodes. Present management practices include (1) preplant fumigation (i.e., methyl bromide or Telone II) that are effective but only for 2-5 years, (2) postplant nematicide application where

recommended, and (3) resistant rootstocks (when available). Current research efforts in the Southeast and California have shifted toward various forms of nonchemical nematode control. Emphasis on nonchemical control is partly due to apprehension about the environmental problems associated with soil fumigation with methyl bromide. As a result of its role in ozone depletion, a ban on the importation and manufacture of methyl bromide in the United States is scheduled for 2005 (Clean Air Act, 1990). Therefore, finding an alternative to chemical control of nematodes is warranted for this pathosystem.

Comment [A36]: Nyczepir

Potatoes suffer from numerous plant diseases including Rhizoctonia stem canker and black scurf. Powdery scab is an increasing problem in the Northeast. This problem is magnified because the powdery scab pathogen serves as the sole vector for the potato moptop virus, recently found throughout the U.S. and Canada. No effective control measures currently exist for either disease problem. Billions of dollars are spent annually in the United States to control potato diseases. Sustainable methods of disease control were evaluated that included rotations, green manures, cover crops, and organic amendments, along with biocontrol agents, microbial inoculants, biostimulants, and novel biocontrol organisms. Specifically, several *Brassica* spp. crops were evaluated in potato rotations for controlling powdery scab and other soil-born diseases while commercially-available biological control organisms such as *Trichoderma virens* and *Bacillus subtilis* were used against stem canker and black scurf. Crop rotations may also play an important role in reducing potato disease.

Comment [A37]: Honeycutt

Crop rotations discovered that reduce reniform nematode populations

A four-year field study evaluating the ability of corn to reduce reniform nematode populations when grown in rotation with cotton was completed by ARS scientists. A crop rotation sequence that effectively reduces reniform nematode populations may help cotton growers maintain high lint yields by keeping this pest population below damaging levels. Of the four rotations evaluated (continuous corn, continuous cotton, cotton-corn-corn-cotton, and corn-cotton-corn-cotton), only the rotation with two consecutive seasons of corn kept nematode populations below damaging levels throughout the cotton season. This rotation increased cotton yields by 194 kg/ha as compared to the continuous cotton plots. At the nematode population levels in our study, a rotation with at least two consecutive years of corn appears to be necessary to achieve nematode suppression and increase cotton yield.

Comment [A38]: Young

Impact: Cultural practices that reduce reniform nematode populations will help growers attain higher yields. Crop rotations with two consecutive years of corn can be used to suppress the pathogen population and simultaneously increase cotton yield. This management strategy should be easily combined with other current and pending methods such as the use of nematicides and the use of resistant or tolerant varieties.

Comment [A39]: Young

Documentation:

Young, L. D., Pettigrew, W. T., Bruns, H. A., Stetina, S. R. 2004. Suppression of reniform nematode populations with cotton-corn rotations. Journal of Nematology. v. 35. p. 354.

Young, L. D., Stetina, S. R., Meredith, W. R., Jr. 2004. Development of cotton germplasm with resistance to reniform nematode. Proceedings of the Beltwide Cotton Conferences. v. 1. p. 427.

Sciumbato, G. L., Stetina, S. R., Young, L. D. 2005. Tolerance of popular cotton varieties to the reniform nematode. Proceedings of the Beltwide Cotton Conferences v. 1.

Reduction of Tomato ringspot virus achieved through crop rotation

Accomplishment: Tomato ringspot virus (ToRSV) is an important disease of red raspberry that is vectored by the dagger nematode, *Xiphinema americanum.* Field trials were conducted to evaluate crop rotation for the management of ToRSV as an alternative to soil fumigation. Rotations with canola or tall fescue, or weed-free fallow were as effective as fumigation with methyl bromide in preventing re-infection of raspberry plants with ToRSV for three years. This research provides a cost-effective management strategy that is readily adaptable to the growers' production system.

Comment [A40]: Linderman

Impact: Tomato Ringspot Virus disease of red raspberry cannot be controlled readily by any means currently because it is nematode vectored. However, reducing the incidence or number of nematodes carrying the virus using precrop plantings of non-host plant species is a reasonable approach to reducing the incidence of the disease on the subsequently planted red raspberry. This method is fully compatible and feasible for growers to use to control this heretofore uncontrollable disease.

Comment [A41]: Linderman

Additional information: The crop rotation research was partially funded partially by a grant from the Northwest Center for Small Fruit Research.

Documentation:

Pinkerton, J. N., Schreiner, R. P., Ivors, K. L., Vasconcelos, M. C. 2004. Effects of *Mesocriconema xenoplax* on *Vitis vinifera* and associated mycorrhizal fungi. Journal of Nematology 36:193-201.

Pinkerton, J. N., and Finn, C. E. 2005. Responses of strawberry species and cultivars to the root lesion and northern root-knot nematodes. HortScience 40:33-38.

Pinkerton, J. N., K. L. Ivors, K. L., Reeser, P. W., Bristow, P. R., and Windon, G. E. 2002. The use of soil solarization for the management of soilborne plant pathogens in raspberry and strawberry production. Plant Disease 86:645-651.

Wheat rotation for reducing ring nematode discovered

Accomplishment: New knowledge pertaining to the use of preplant wheat rotation as an alternative to chemical control of the ring nematode was investigated. It was determined that preplant wheat suppressed ring nematode as well as methyl bromide fumigation during orchard establishment. However, interplanting wheat under established peach trees did not suppress the nematode at all. Wheat rotation technology has been successfully transferred to the peach industry and is being used. This technology also has been incorporated in the 2004 SE Peach, Nectarine, and Plum Pest Management and Culture Guide. Utilization of selected rotations in conjunction with an improved rootstock for suppression of nematodes and incidence of peach-tree-short-life (PTSL) is the long-term objective.

Comment [A42]: Nyczepir

Impact: The impact of the wheat rotation and rootstock findings has provided the peach industry with alternative nonchemical control strategies for managing nematode-associated disease problems on peach.

Comment [A43]: Nyczepir

Additional information: The wheat rotation research involved a collaborative effort with scientists from the University of Georgia and was supported by outside funding from the Georgia Agricultural Commodity Commission for Peaches. The wheat rotation collaborators had expertise in areas other than Nematology (i.e., extension, wheat management & disease control) that contributed to the success of this accomplishment.

Comment [A44]: Nyczepir

Documentation:

Nyczepir, A. P. and Bertrand, P. F. 2000. Preplanting bahia grass or wheat compared for controlling *Mesocriconema xenoplax* short life in a young peach orchard. Plant Disease 84:789-793.

Nyczepir, A. P. Field evaluation of nonchemical alternatives for control of ring nematode on peach. 2003. Proceedings of the Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, San Diego, CA, Nov. 3-6, 2003, p. 118, 1-2.

Nyczepir, A. P., Okie, W. R., and Beckman, T. G. 2004. Creating a short life site for *Prunus* rootstock evaluation on land with no innate *Mesocriconema xenoplax* population. HortScience 39(1): 124-126.

Guardian peach rootstock widely accepted tool for reducing peach root diseases

Accomplishment: Guardian rootstock was found to survive better than the standard peach rootstock on PTSL sites in the presence of ring nematode. Guardian also exhibited root-knot nematode resistance to certain species, but not all. Guardian rootstock technology has been successfully transferred to industry and is now commercially available. Since 2002, ca. 80% of peach trees delivered to commercial growers in the Southeast have been propagated on Guardian, displacing all standard commercial rootstocks previously used by this industry. As of 2003, over 10 million Guardian seed (1.4 million in 2003 alone) has been distributed to commercial nurseries in the Southeast. Plant Variety Protection Patent #9400013 was granted on June 14, 2001, for Guardian (BY520-9) rootstock.

Comment [A45]: Nyczepir

Impact: The impact of the rootstock findings has provided the peach industry with alternative nonchemical control strategies for managing nematode-associated disease problems on peach.

Comment [A46]: Nyczepir

Additional information: The rootstock research involved a collaborative effort with scientists from ARS (Byron, Georgia), Clemson University, and Spain, and was supported by outside funding from the SC Commission for Peaches. Rootstock collaborators had expertise in the areas of rootstock breeding and horticulture. A collaborator from Spain had an expertise in root-lesion nematode culturing.

Documentation:

Nyczepir, A. P. and Beckman, T. G. 2000. Host status of Guardian peach rootstock to *Meloidogyne* sp. and *M. javanica*. HortScience 35:772.

Nyczepir, A. P. and Pinochet, J. 2001. Assessment of Guardian peach rootstock for resistance to two isolates of *Pratylenchus vulnus*. Journal of Nematology 33:302-305.

Brassica cover crops and antagonists found to reduce potato diseases

Accomplishment: Commercially-available biological control organisms such as *Trichoderma virens* and *Bacillus subtilis* were found to reduce Rhizoctonia stem canker on potato by 37-75% and black scurf by 11-20%, while increasing potato yield by 15-20%. Greenhouse and field tests demonstrated that *Brassica* cover crops can significantly contribute to controlling powdery scab and other soil-born diseases.

Comment [A47]: Honeycutt

Impact: These practices/amendments have significant potential for improving disease control and reducing pesticide inputs.

Documentation:

Larkin, R.P. 2002. Effects of different 3-yr cropping systems on Rhizoctonia canker and black scurf of potato in northern Maine, 2000 and 2001. Biological and Cultural Tests. v.17. p.PT07.

Economic feasibility of potato crop rotations assessed

Accomplishment: An economic analysis was conducted of crop rotations that suppress disease in potato. Rotation with canola resulted in lower disease incidence, higher potato yield, and higher net income compared to other, less disease suppressive rotations. However, rotation length and sequence were found to influence the efficacy of these systems and their economic returns.

Comment [A48]: Honeycutt

Impact: These results provide potatogrowers with guidance for enhancing profitability through disease suppression.

Additional information: The Maine Potato Board has contributed funding to evaluate potential controls for powdery scab. This has facilitated performing on-farm research that has identified the potential of *Brassica* crops for controlling powdery scab and other soil-born diseases.

Documentation:

Larkin, R.P. 2003. Characterization of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles. Soil Biology & Biochemistry 35:1451-1466.

Discovery Area 5: Crop Health and Cover Crops Impact Disease Development

Cultural control options for reducing plant diseases are not always easily categorized. A variety of cultural control discoveries are presented here that range from improving crop health by managing soil pH or removing soil to expose plant collars to air, to managing cover crops to reduce both pathogen and pathogen vector. For instance, cacao,

Comment [A49]: Schisler

vegetables, and other crops, which may be alternatives to illicit narcotic crops, are susceptible to many fungal diseases. Among these diseases are those caused by *Phytophthora* spp. (damping-off, root, crown and pod rots, and blights) on cacao and many other crops, and those diseases specifically on cacao caused by *Crinipellis perniciosa* (witches' broom and pod rot) and *Moniliophthora rorei* (frosty pod rot). Successful cultivation of alternative crops is dependent on managing these diseases using integrated disease management strategies, preferably in a sustainable crop production system. A systems approach to disease control will enable the development of sustainable crop production strategies that will allow management of disease outbreaks and reduction in crop disease losses. Along this line, studies to improve nutrient recovery and utilization efficiency of plants by managing soil pH in acid infertile tropical soils were undertaken. Studies on the effect of soil nutrition on microbial communities in tropical soils were also initiated.

Comment [A50]: Bailey

Studies on the control of grapevine diseases was the main emphasis of another project, with a focus on developing cultural and biological treatments that either compliment or replace chemical treatments. Challenges to this research include incomplete knowledge of grapevine disease epidemiology and the need for methods to assess efficacy of control treatments. A thorough knowledge of disease epidemiology is crucial for developing effective disease control practices, in order to best target the weakest point in the pathogen's life cycle and/or to prevent dispersal of infectious propagules. Once a thorough knowledge of the epidemiology of a disease is achieved, then methods are needed that allow for accurate evaluation of control practices for the disease. Additionally, it is difficult to adapt treatments that were originally developed for other crops, as the increasingly popular use of deficit wine grape management practices to intentionally reduce grapevine vigor and yield (e.g. vineyard establishment on marginal soils and deficit irrigation) is atypical of most perennial cropping systems.

Comment [A51]: Baumgartner

Abiotic factors of importance to managing tropical crops discovered

Accomplishment: Reducing soil acidity constraints and improving light quality is crucial to the productivity and nutrient use efficiency of cacao and cover crops. Inter-intra specific differences in cacao and tropical legume cover crops for tolerance to abiotic stresses, nutrient use efficiency and growths were identified. Cover crops were successful in controlling weeds in cacao plantations.

Comment [A52]: Bailey

Impact: Reducing soil acidity constraints and improving light quality improved productivity and nutrient use efficiency of cacao and cover crop. Such finding will help to design better management practices for perennial tropical crops. The cover crops tested were sensitive to low light intensity which reinforced the importance of shade and fertilizer management to achieve the maximum potential benefit of cover crops.

Additional information: Research by ARS scientists was carried out through SCAs with Brazilian Institutes (CEPLAC/CPEC, Almiranti Cacao Research Center) and in Peru (Tropical Crop Institute, ICT) and non funded Cooperative agreement with State

University of Santa Cruz (UESC) and South Bahia Eciligical Institute (IESB) in Bahia Brazil.

Comment [A53]: Bailey

Documentation:

Baligar, V. C., N. K. Fageria, H. Eswaran, M. J. Wilson and Z. He. Nature and properties of red soils of the world. p.5- 30. In. M. J. Wilson, Z. He and X. E. Yang (eds.) The Red Soils of China: Their Nature, Management and Utilization Kluwer Academic Pub, Dordrechet, The Netherlands. 2004.

Yang, X., W. Wang, Z. Ye, Z. He and V. C. Baligar. Physiological and genetic aspects of crop plant adaptation to elemental stress in acid soils .p. 179- 232.In. M. J. Wilson, Z. He and X. E. Yang (eds.) The Red Soils of China: Their Nature, Management and UtilizationKluwer Academic Pub, Dordrechet, The Netherlands. 2004.

Techniques for mitigating grapevine diseases developed

Accomplishment: ARS scientists developed a method for evaluating the efficacy of control treatments for Armillaria root disease of grapevine and, then, developed a new cultural treatment known as 'root collar excavation' to control the disease. Root collar excavation significantly improved yields of symptomatic grapevines and increased grape cluster weights to sizes comparable to those of healthy grapevines.

ARS scientists modified a cultural control practice for Pierce's disease of grapevine in the North Coast of California known as 'riparian revegetation management', a technique that involves replacement of hosts of both the pathogen, *Xylella fastidiosa*, and the vector, *Graphocephala atropunctata* (blue-green sharpshooter) with non-hosts. Two invasive weeds, *Vinca major* (periwinkle) and *Rubus discolor* (Himalayan blackberry) were discovered to be important reservoirs of *X. fastidiosa*. In contrast, the native riparian plants studied, *Rubus ursinus* (California blackberry) and *Sambucus mexicana* (blue elderberry), were not important reservoirs of *X. fastidiosa*. As California blackberry and elderberry may not be important reservoirs of *X. fastidiosa*, efforts expended in removing these species may not be repaid with a reduction in disease incidence.

Comment [A54]: Baumgartner

Impact: Results of ARS research on root collar excavation have scientific significance and economic significance. The methodology we developed to evaluate control practices for Armillaria root disease gives scientists a tool to test other control practices. Root collar excavation is currently the only postinfection control practice (chemical or non-chemical) for Armillaria root disease, which attacks approximately 500 species of woody plants, worldwide. Root collar excavation improves yields of infected grapevines, which, in turn, results in higher profit, as grapes are sold by weight. Results of ARS research on Pierce's disease have economic and environmental significance. Grape-growers that manage riparian vegetation to control Pierce's disease can focus on clearing fewer plant species, thereby cutting labor costs associated with plant removal. By removing fewer plants in riparian areas adjacent to vineyards, there is less disruption of wildlife habitat. Removing invasive weeds may help restore riparian areas to a more natural condition.

Comment [A55]: Baumgartner

Additional information: Outside funding from competitive grants that contributed to this research were provided by the following organizations/agencies: American Vineyard Foundation, Viticulture Consortium, and California Department of Food & Agriculture. Collaborations with the Department of Plant Pathology, University of California, Davis, contributed to research on identity and distribution of *Armillaria* species in California

Documentation:

Baumgartner, K., Rizzo, D.M. 2001. Distribution of *Armillaria* species in California. Mycologia. v. 93 p. 821-830.

Baumgartner, K., Rizzo, D.M. 2001. Ecology of *Armillaria* spp. in mixed-hardwood forests of California. Plant Disease. v. 85 p. 947-951.

Baumgartner, K., Rizzo, D.M. 2002. Spread of Armillaria root disease in a California vineyard. American Journal of Enology and Viticulture v. 53 p. 197-203.

Baumgartner, K. 2004. Root collar excavation for postinfection control of Armillaria root disease of grapevine. Plant Disease v. 88 p. 1235-1240.

Pathogen Biology, Genetics, Population Dynamics, Spread, and Relationship with Hosts and Vectors Component

Background: Pathogen biology, genetics, population dynamics, spread, and relationship with hosts and vectors are often not adequately understood. Understanding the processes that take place during disease development often leads to methods that interrupt the life cycle of pathogens and prevent disease. Similarly, understanding how pathogens move from plant to plant or within harvested commodities, learning how they survive lacking host material, and knowing how they interact with their environment can suggest points where they may be susceptible to control. Knowledge of pathogen survival on plant parts and seeds is needed to develop methods to reduce the spread of diseases, both domestically and internationally.

Dicovery Area I: **An Understanding of the Pathogen Biology, Virulence Determinants of Pathogens, and Genetics of the Pathogen**.

Fusarium Head Blight

Background: *Gibberella zeae* (anamorph *Fusarium graminearum*) causes Fusarium head blight or scab of wheat and barley. In the last decade, *G. zeae* has caused destructive epidemics on wheat and barley in the United States and Canada with cumulative losses estimated at greater than three billion dollars. The goals of this project are to detect new resistance genes, develop molecular markers for resistance genes, and to add to our basic knowledge of the pathogen.

Accomplishments: ARS developed the first genetic map for *Gibberella zeae*, the major fungal pathogen of Fusarium head blight of wheat that produces several mycotoxins harmful to humans and domesticated animals. In collaboration with colleagues in Germany, ARS identified chromosomal regions associated with pathogenicity and aggressiveness on a genetic map of *G. zeae*. One major gene that controls pathogenicity in the region that controls toxin amount was also mapped. ARS mapped one or two minor genes that control aggressiveness in the region that controls toxin type (nivalenol or deoxynivalenol). Progeny that produced deoxynivalenol were, on average, about twice as aggressive as were those producing nivalenol.

Impact: The genetic map has been used in genetic diversity studies and to validate and align the genomic sequence of *Fusarium graminearum* (http://www.broad.mit.edu/annotation/fungi/fusarium/markers.html). The alignment of linkage groups and supercontigs revealed that there are four chromosomes in this fungus. The genetic analysis of aggressiveness in this interlineage cross-revealed rather simple inheritance suggesting that relatively few loci for pathogenicity or aggressiveness differ between lineage 6 and 7. Therefore, the risk of sexual recombination between these two lineages may be lower than expected.

Additional Information: The work was done in collaboration with Kansas State University, the University of Northern Iowa, and the University of Hohenheim, Stuttgart, Germany.

Documentation:

Cumagun, C. J. R.; Bowden, R. L.; Jurgenson, J. E.; Leslie, J. F., and Miedaner, T. 2004. Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat Phytopathology 94:520-526.

Jurgenson JE; Bowden RL; Zeller KA; Leslie JF; Alexander NJ, and Plattner RD. 2002. A genetic map of *Gibberella zeae* (*Fusarium graminearum*). Genetics 160:1451-1460.

Zeller, K. A.; Bowden, R. L., and Leslie, J. F. 2003. Diversity of epidemic populations of Gibberella zeae from small quadrants in Kansas and north Dakota. Phytopathology 93(7):874-880.

Zeller, K. A.; Bowden, R. L., and Leslie, J. F. 2004. Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States. Mol. Ecol.; 13:563-571.

Lee, J., J. E. Jurgenson, J. F. Leslie and R. L. Bowden. 2004. The alignment between physical and genetic maps of *Gibberella zeae* (abstract). Phytopathology 94:S58.

Pseudomonas syringae

Background: Effector proteins are used by bacteria to disrupt plant metabolism during infection. Effectors are introduced into plant cells using a specialized injection apparatus (the type III secretion system) that is employed by a wide variety of plant and animal pathogens.

Accomplishments: ARS developed an integrated laboratory-computational approach to identify effector proteins in the DNA sequence of bacterial pathogens in general, and in *Pseudomonas syringae* in particular. This method has been used to identify 23 previously unknown effector proteins in the *P. syrinage* genome. The new information is being used by plant pathologists to understand the molecular basis of pathogenesis and disease resistance.

Impact: The identification of new effectors is a major step toward a complete inventory of these proteins in a plant pathogen and will result in an increased understanding of how bacteria interact with their plant hosts. Two patent applications arising from this work are pending: “Methods of Identifying Putative Effector Proteins (60/348,061) and “Pseudomonas Avr and Hop Proteins, Their Encoding Nucleic Acids, and Use Thereof” (60/380,185). Our work in identifying type III secretion system substrates, including effectors, chaperones, and helpers, has had a significant impact in several areas of molecular pathogenesis. For example, two groups have characterized the biochemical

role of HopPtoD2 as a tyrosine phosphatase that modulates MAPK pathways involved in programmed cell death in plants. Other workers have confirmed cysteine protease activity in AvrPphB and a previously characterized effector in *Yersina pestis*, YopT. This has resulted in the definition of a new family of proteins with widespread importance in bacterial pathogenesis. Also, a number of laboratories have confirmed that *P. syringae* effectors modulate programmed cell death pathways in plants, fungi, and even animals. Some effectors appear to trigger programmed cell death while others appear to prevent it. Finally, the existence of a large collection of confirmed effectors in *P. syringae* have enabled other laboratory workers to identify several new R-genes (disease resistance) in *Arabidopsis thaliana*.

Additional Information: This work was a collaborative effort between ARS and the Department of Plant Pathology, Cornell University, and was supported by a Specific Cooperative Agreement and a grant from the National Science Foundation Plant Genome Program.

The global measurement of mRNA abundance is an important part of understanding how genes are expressed in response to changes in the environment and other factors. In collaboration with the Boyce Thompson Institute, we compared mRNA abundance in hrpL mutant and wild-type strains using a DNA microarray containing 5800 ORF-specific PCR products corresponding to genes in the DC3000 genome. These experiments will help us understand the structure of the hrpL regulon and its role in pathogenesis. Genes in sequenced bacterial genomes are typically identified using computational methods rather than direct evidence from laboratory experiments. Using a high-throughput mass spectrometry-based proteomics screen, we have identified 1500 proteins in DC3000. The data reveal that approximately 15 to 20% of proteins do not correspond to the published annotation for *P. syringae* DC3000 and also reveals genes that were not included when the genome was first annotated. This information is essential if gene regulation is to be understood at the global level.

Documentation:

Buell CR, Joardar V, Lindeberg M, Selengut J, Paulsen IT, Gwinn ML, Dodson RJ, Deboy RT, Durkin AS, Kolonay JF, et al. 2003. The complete genome sequence of the Arabidopsis and tomato pathogen Pseudomonas syringae pv. tomato DC3000. Proc Natl Acad Sci U S A. Sep 2;100(18):10181-6.

Fouts DE, Abramovitch RB, Alfano JR, Baldo AM, Buell CR, Cartinhour S, Chatterjee AK, D'Ascenzo M, et al.. 2002. Genomewide identification of Pseudomonas syringae pv. tomato DC3000 promoters controlled by the HrpL alternative sigma factor. Proc Natl Acad Sci U S A. Feb 19;99(4):2275-80.

Collmer A, Lindeberg M, Petnicki-Ocwieja T, Schneider DJ, Alfano JR. 2002. Genomic mining type III secretion system effectors in Pseudomonas syringae yields new picks for all TTSS prospectors. Trends Microbiol. Oct;10(10):462-9.

Petnicki-Ocwieja T, Schneider DJ, Tam VC, Chancey ST, Shan L, Jamir Y, Schechter LM, Janes MD, Buell CR, Tang X, Collmer A, Alfano JR. 2002. Genome wide identification of proteins secreted by the Hrp type III protein secretion system of Pseudomonas syringae pv. tomato DC3000. Proc Natl Acad Sci U S A. May 28;99(11):7652-7657.

Phytoplasmas

Background: Phytopathogenic spiroplasmas and phytoplasmas are cell wall-less bacteria that parasitize insect vectors and plant hosts, causing economically important plant diseases in crops such as potato, strawberry, grapevine, jujube, walnut, bean, carrot, peach, cherry, pear, and almond. Although these micro-parasites impact agriculture significantly, little is known about the fundamental biology of the pathogens and the mechanisms of their pathogenesis. This project is based on the hypothesis that knowledge gained from structural and functional genomics will make it possible to devise improved and novel means for genus-level, species-level, and virulent strain-level detection and identification of the pathogens, including invasive species. Comparative genomic studies will lead to identification of potential genes useful for detection and identification of the pathogens at various taxonomic levels. The genome information and new knowledge of bacterial pathogenesis will promote fundamental and applied research on disease control.

Accomplishments: ARS sequenced and annotated entire genome of *Spiroplasma kunkelii*, the helical, motile, and cell wall-less bacterium that causes devastating corn stunt disease in maize and assembled major metabolic pathways and functional systems that operate in the pathogen and discovered new genes and system components that may be involved in pathogenesis. ARS also functionally characterized selected *S. kunkelii* genes that are potential molecular targets for disease control. On the front of phytoplasma genome research, ARS discovered inactive folate biosynthesis genes and theorized that folate biosynthesis genes have undergone lineage-specific decay. Further, ARS discovered novel insertion sequences (IS), which represent a key component in phytoplasma chromosomal rearrangements and may constitute a major factor in disease symptom expression.

Impact: Spiroplasmas parasitize insects, ticks, and plants; some induce pathology in vertebrate animals, and some have been linked to human diseases. The accomplishment provided information-dense publications containing concepts and theories impacting plant pathology, insect pathology, and animal and human medicine. It formed a basis for new theories of spiroplasma biology and evolution. The information is being used by The Institute of Genome Research, Maryland, as a key element in work to design and construct a minimal cell, a major goal to benefit fundamental science and human health. The information is also being used by Institut National de la Recherche Agronomique, France, to help sequence the genome of a related pathogen *S. citri*. The much-needed information is also being used by California Department of Agriculture, in collaboration with ARS, to develop real-time PCR protocols for early detection of corn stunt epidemics; in California, an outbreak of the disease in 2002 caused damage exceeding $5

million in Kings County alone. These fundamental discoveries of folate biosynthesis pseudogenes and IS elements in phytoplasma genomes have already led to new hypothesis on mollicutes evolution, niche adaptation, and emergence of new pathogens. On the practical aspect, these new discoveries have improved strain/species differentiation, classification, and will lead to development of new tools for pathogen detection and identification.

Documentation:

Zhao, Y., Hammond, R.W., Jomantiene, R., Dally, E.L., Lee, I.-M., Jia, H., Wu, H., Lin, S., Zhang, P., Kenton, S., Najar, F.Z., Hua, A., Roe, B.A., Fletcher, J., and Davis, R.E. 2003. Gene content and organization of an 85-kb DNA segment from the genome of the phytopathogenic mollicute *Spiroplasma kunkelii*. Mol. Genet. Genomics 269:592-602.

Davis, R.E., Jomantiene, R., Zhao, Y., and Dally, E.L. 2003. Folate Biosynthesis pseudogenes, Ψ*folP* and Ψ*folK*, and an O-sialoglycoprotein endopeptidase gene homolog in the phytoplasma genome. DNA and Cell Biology 22:697-706.

Zhao, Y., Hammond, R.W., Lee, I.-M., Roe, B.A., Lin, S., and Davis, R.E. 2004. Cell division gene cluster in *Spiroplasma kunkelii*: functional characterization of *ftsZ* and the first report of *ftsA* in mollicutes. DNA and Cell Biology 23:127-134.

Zhao, Y., *Wang, H.*, Hammond, R.W., Jomantiene, R, *Liu, Q.*, Lin, S., Roe, B.A., and Davis, R.E. 2004. Predicted ATP-binding cassette systems in the phytopathogenic mollicute *Spiroplasma kunkelii*. Mol. Genet. Genomics 271:325-338.

Lee, I.-M., Zhao, Yan, Bottner, K.D. 2004. Identification of insertion sequence (IS)-like elements in the aster yellows group phytoplasmas. FEMS Microbiology Letters 242:353-360 online www.science@direct.

Phytophthora

Background: Late blight**,** caused by *Phytophthora infestans*, is the most destructive disease of potato and tomato worldwide. Novel strains of *P. infestans* have evolved which possess the potential to overcome genetic resistance introgressed by conventional breeding from wild species into commercial varieties. Worldwide migrations and sexual recombination have introduced new, more virulent strains. Development of new, rapid molecular protocols to aid in identification of novel, more virulent isolates is needed to monitor the origins and spread of pathogen, and to characterize variability. Alternative strategies for late blight control, such as molecular engineering, are also needed to address the disease problems resulting from new pathogen isolates.

Accomplishments: Genetic variation was examined in a study evaluating over 300 strains of *P. infestans* that were collected in Taiwan during an eight-year period. During this timeframe, a fungicide resistant strain of the pathogen appeared in the outdoor tomato crops grown in the highlands. DNA fingerprinting was used to characterize

pathogen population diversity. By using unique DNA sequences as molecular markers, ARS was able to demonstrate greater levels of genotypic diversity in the new populations of *P. infestans*, and that population structures differed from one location to another. In 2003, a new disease was detected on petunia transplants at a glasshouse in central Maryland that provides flowers and bedding plants to local markets. This leaf blight on petunia was caused by *P. infestans*. This is the first time that *P. infestans* was isolated and characterized on greenhouse grown petunias in the state of Maryland. This discovery indicates that floral and bedding plants could harbor this pathogen, presenting a new potential host of the pathogen that could spread to potatoes, tomatoes, and other solanaceous crops. In 2004, ARS reported the isolation and characterization of *P. infestans* strains from another Solanaceous host, hairy nightshade, growing within and around fields of blighted potatoes in Maine.

In addition, a new tool for pathogen characterization was developed based on cloning of the gene for a widely used isozyme marker, glucose-6-phosphate isomerase. Application of this tool provided evidence that a new isolate of *P. infestans*, infecting tomato tree in Ecuador, may have arisen from out-crossing with *P. erythroseptica*.

Impact: The major impact of understanding pathogen diversity will be to ensure that cultivars resistant to late blight have nonspecific (horizontal) resistance, not limited to virulence genes present in the current, local populations of the pathogens. Examination of field isolates and comparison with archival strains provides information on the population structure over time. This information is vital if we are to continue to make effective use of disease resistance genes in late blight control strategies. This information directly impacts tomato and potato breeding programs, and plays an important role in trade issues involving pathogen presence and origin. After identification of new hosts, potato growers were made aware that both weed and cultivated Solanaceous species can be infected with *P. infestans* and may serve as clandestine reservoirs of inoculum.

Gene characterization in *P. infestans* has direct impact on studies of the genus *Phytophthora*. Genes found in *P. infestans* have homologues in other devastating pathogens such as *P. sojae* and *P. ramorum*. Gene characterization can also identify gene flow between species that may cause the appearance of new pathogens. Molecular strategies for *P. infestans* control not only provide new ways to manage a disease of worldwide importance, these strategies may directly influence control of other *Phytophthora* species.

Additional Information: To ascertain the present status of the Taiwanese population of *P. infestans*, we are currently collaborating with the Asian Vegetable Research and Development Center where isolates of the pathogen were collected and maintained. The goal is to determine if knowledge gained through characterization of isolates collected from natural infections on potato and tomato hosts may provide insights into the population dynamics of this pathogen in the United States.

Documentation:

Deahl, K. L., Cooke, L. R., Black, L., Wang, T., Perez, F., Moravec, B., Quinn, M. and Jones, R. 2002. Population changed in *Phytophthora infestans* in Taiwan associated with the appearance of resistance to metalaxyl. Journal of Pest Management Science 58:951-958

Ospina-Giraldo, M.D. and Jones, R.W. 2003. Characterization of the glucose-6-phosphate isomerase gene in *Phytophthora infestans* reveals the presence of multiple alleles. Fungal Genetics and Biology 40:197-206.

Deahl, K. L. and Fravel, D. 2003. Occurrence of leaf blight of petunia, caused by *Phytophthora infestans* in Maryland. Plant Disease 87:1004

Jones, R.W., Ospina-Giraldo, M.D. and Clemente, T. 2004. Prosystemin-antimicrobial-peptide fusion reduces tomato late blight lesion expansion. Molecular Breeding 14: 83-89.

Potato Scab

Background: Potato common scab is a chronic problem, affecting potato quality and therefore marketable yield. Scab has been rated by growers as the fourth most significant potato disease, and there are limited options for chemical or biological control. Common scab incidence and severity vary from year to year and location to location, although we lack knowledge about the reasons for variability. Little information is available on variation within the scab pathogen populations in the field or variation in scab resistance in potato germplasm. The most reliable control of common scab would be the availability of resistant potato varieties, but no true resistance to common scab has been identified so far. Reliable information on pathogen and plant variability is essential for identifying potential disease resistance markers that can be used in selecting more resistant potato varieties, and for developing disease management strategies to minimize losses to growers.

Accomplishment: ARS demonstrated that scab symptoms are not seen at low pathogen densities, and that lesion type severity and tuber surface area affected increase as the pathogen inoculum increases over several orders of magnitude. ARS concluded that fluctuations in pathogen population density are important components of variation in disease severity. In addition, ARS documented variation in pathogen virulence and aggressiveness within a large collection of isolates made from scabby potatoes from many regions of the United States. Molecular characterization of numerous isolates has enabled development of molecular fingerprints for individual isolates or groups of isolates. This information can be used to trace the origins and spread of common scab in potato growing areas.

Impact: The impact of these research results comes from improved scientific methods for assessing genetic resistance to potato common scab. Since it has been demonstrated that the severity of common scab is dependent on the density of the pathogen inoculum as well as the aggressiveness of the pathogen strain, it is essential to standardize the pathogen inoculum when assessing resistance to common scab in commercial potato varieties or breeding lines. This information can be used by breeders and plant pathologists, when selecting disease-resistant materials and assessing resistance among cultivars. Measurements of pathogen load could be applied to assaying the likelihood of a serious scab problem causing economic losses in specific field locations and years. Improved diagnostic reagents will shed light on pathogen population dynamics and allow us to trace the movement of new and existing pathogen strains.

Additional Information: Some outside funding and collaboration have been contributed by USDA/ARS Cooperative Potato Research Program in a project entitled "Characterization of Potato Cultivar Response to Powdery and Common Scab." The investigators in this project include USDA/ARS, Beltsville, Maryland; USDA/ARS, Aberdeen, Idaho; theUniversity of Minnesota, St. Paul, Minnesota; the Pennsylvania State University, University Park, Pennsylvania; the Colorado State University, Center, Colorado; and the University of Idaho, Aberdeen, Idaho. This project was funded for 2 years, and enabled field-testing of approximately 20 potato genotypes per year for common scab susceptibility at three different sites, one in Maine, one in Minnesota, and one in Idaho. Differences in susceptibility were seen for the same genotype when grown in different locations, implying that scab pathogen populations probably differ at the three sites. In addition to assessment of scab susceptibility in potato plant material, collaborators have supplied materials (scabby potatoes) for isolation and characterization of the variation in scab pathogens from these three and other areas of the United States.

Documentation:

Wanner, L.A. 2004. Field isolates of *Streptomyces* differ in pathogenicity and virulence on radish. Plant Disease 88:785-796.

Virus-Based Vectors

Background: Plant diseases caused by cellular and sub-cellular pathogens, including fungi, bacteria, phytoplasmas, viruses, and viroids, are an important component of agricultural systems that affect crop yield and quality, and the development of improved disease control strategies is a continuing and long-term goal of ARS. The development of resistant plant cultivars by conventional breeding or transformation and regeneration techniques requires significant time and effort, and the new transformation technology is not applicable to all plant species. These factors hinder the rapid evaluation of sequences for disease control. Plant virus-based vectors as transient gene expression systems are an attractive alternative to conventional breeding and transformation technology. Gene delivery systems employing plant viruses are being developed and used to optimize expression and cellular and sub-cellular targeting of foreign genes and their protein products and will be used to test efficacy of these genes for controlling specific pathogens in potato, tomato, vegetables, and sugarbeet.

Accomplishment: Sugar beet is difficult to transform, and the transformation technology that exists is owned by the industry. These factors hinder the rapid evaluation of sequences for disease control; plant virus-based vectors offer and attractive alternative for the expression of foreign genes. Beet mosaic virus (BtMV), a member of the economically important Potyvirus group of plant viruses, is being developed as a viral-based transient expression vector for sugar beet. ARS determined the nucleotide sequence of the complete viral genome and have found that it is a distinct virus species. This information allowed ARS to construct full-length infectious copies of the virus which will be further engineered to express antimicrobial peptides in sugar beet to assess their protective properties. The development of a BtMV vector will allow ARS to rapidly evaluate antimicrobial genes for control of major diseases of sugar beet, including a novel bi-component antimicrobial protein that has been shown to be active against the *Clavibacter* bacterium.

The development and use of a transient assay system using plant viral-based vectors to assess the potential of foreign gene sequences for disease control of cellular and subcellular pathogens and for production of pharmaceuticals in a wide range of host plants is an attractive alternative to transgenic technology. ARS has successfully developed the wide host range virus, Cucumber mosaic virus (CMV), into a viral-based vector for extended host range expression of foreign genes. The expression strategy is based on the use of protein fusion and a self-cleaving peptide to express foreign genes while retaining systemic movement of the virus. This expression strategy will allow us to exploit CMV for fundamental research targeted toward disease control strategies and for expression of pharmaceutical proteins in a wide range of plants.

Impact: Novel plant virus-based vectors for expression of foreign gene sequences in plants will reduce or eliminate insect transmission of viral-based vectors by site-directed mutagenesis of amino acids known to be important for insect transmission. These vectors will optimize expressions and cellular targeting of foreign genes controlling specific pathogens. They will also be used to test new technologies and products to control diseases of animals. Improved diagnostic reagents will help protect domestic crops from existing and foreign pathogens. Reducing losses to crops by control of plant diseases is the long term objective.

Additional Information: Viral-based vectors were also developed to test new technologies and products to control diseases of animals. Existing animal disease control strategies are often ineffective or their implementation is problematic. Contaminated reagents and time consuming administration of vaccines contribute to the problem. In recent years, the use of plants for the production of bio-engineered vaccines, antibodies, and enzymes has shown promise. The advantages of plants as production systems include safety, affordability, rapid turn-around time, rapid scale up, ease of storage and purification, among others. The expressed protein can also be fed directly as plant material to animals. For the expression of candidate biomedicals in plants, plant viruses offer the advantages of speed of product development, flexibility, and high levels of gene expression. We are using our expertise in plant virology to develop new technologies

and products for prevention, treatment, and control of various diseases of animals and to extend the range of plants, including fruit and vegetable crops that can be used as "factories."

Accomplishment: ARS developed a modified plant virus to produce, in plants, a bovine therapeutic protein for prevention of a serious disease in cattle. Transient expression of the modified virus in *Nicotiana benthamiana* plants resulted in a biologically active protein. An Invention Report describing our production of a bovine therapeutic protein in plants has been filed with the Office of Technology Transfer, and we are awaiting a determination of patentability.

Impact: Viral-based vectors will be developed to test new technologies and products to control diseases of animals, including engineered vaccines and therapeutic proteins. Improved diagnostic reagents and vaccines for control of animal diseases will benefit producers and processors.

Additional Information: A Specific Cooperative Agreement with the Biotechnology Foundation, Inc. facilitates cooperative research on the development of low-cost, safe and effective biomedicals in plants. A specific example of this cooperation is the development of improved diagnostic reagents for bovine diarrheal virus (BVDV), the largest source of viral-associated loss in the U.S. beef and dairy industries. BVDV contamination is a problem for diagnostic laboratories and hinders disease control. We have expressed BVDV surface antigens as fusions with plant virus proteins, and used the purified virus particles to prepare reagents for improved BVDV diagnostic kits.

Through State Department funding, collaboration with scientists in the ARS-Former Soviet Union Scientific Cooperation Program has resulted in the development of novel, oral, plant-based vaccines against human viral diseases, including hepatitis B and HIV by using engineered plants to express viral antigens.

Documentation:

Hammond, R., Nemchinov, L.G., Zhao, Y. 2004. Mutation and recombination may limit the use of cucumber mosaic virus as a virus-based gene expression vector. American Phytopathological Society. 94(S):912

Nemchinov, L.G., Zhao, Y., Hammond, R. 2004. Examination of the utility of plant virus capsid protein fusions to produce Newcastle disease virus vaccine antigens. Meeting Proceedings. p.64.

Salyaev, R. K., Rekoslavskya, N. I., Posdnyarkov, S. G., Ryzhova, T. S., Nesterov, A. E., Sumtzova, V. M., Pakova, N. V., Mishutina, U. O., Kopytina, T. V., Hammond, R., Shchelkunov, S, N, 2004. The immunization of mice by feeding of fruits of transgenic tomato containing the integrated gene TBI-HBS encoding the synthesis of antigenic protein epitopes of Hepatitis B virus and HIV. Meeting Proceedings p.68.

Zhao, Y., Hammond, J., Tousignant, M. E., Hammond, R. 2000. Development and evaluation of a complementation-dependent gene delivery system based on Cucumber mosaic virus. Archives of Virology. v. 145 p. 2285-2295. 2000.

Zhao, Y., Owens, R. A., Hammond, R. W. 2001. Use of a vector based on Potato virus X in a whole plant assay to demonstrate nuclear targeting of Potato spindle tuber viroid. Journal of General Virology. v. 82 p.1491-1497.

Hammond, J., Hammond, R. 2003. The complete nucleotide sequence of Bean yellow mosaic virus isolate BYMV-GDD and comparison to other potyviruses. Archives of Virology. v. 148 p. 2461-2470.

Nemchinov, L.G., Hammond, J., Jordan, R.L., Hammond, R. 2004. The complete nucleotide sequence, genome organization, and specific detection of Beet mosaic virus. Archives of Virology. v. 149 p. 1201-1204.

Salem, N., Mansour, A., Al-Musa, A., Al-Nsour, A., Hammond, R. 2004. Identification and partial characterization of Prunus necrotic ringspot virus on stone fruits in Jordan. Journal of Plant Pathology. v. 86 p. 83-87.

Xyllella

Background: Exotic plant pathogens are a leading threat to U.S. agriculture, and impacts viticulture, almond, citrus, tree crops, and woody ornamentals, among other crops. *Xylella fastidiosa* has emerged in the last decade from obscurity and is among the greatest threats to both the U.S. citrus and grapevine industries. Citrus is particularly vulnerable to exotic pathogens, because the trees are clonal, the industry is global, and the pathogens are mobile. These factors make breeding for resistance impractical, create a great diversity of pathogens, and make their exclusion from the United States impossible. These pathogens must be characterized in advance of their arrival into the United States to better understand their biology and relationships with other pathogens in order to facilitate control and/or eradication programs. This work can best be done at a secure location far removed from the domestic citrus industry to provide a level of biological security that physical methods alone cannot. Such research may also reveal similarities to pathogens of domestic concern, and lead to common strategies for control and management of these diseases.

Accomplishment: *Xylella fastidosa* is difficult to grow and at the beginning of this project no genetic systems had been developed for this organism. The diversity of strains of the organism and the mechanism(s) used by the pathogen to colonize plant tissue and induce disease were also poorly understood. ARS has made mutants of *X. fastidiosa* labeled with green fluorescent protein and used these mutants to study host pathogen interactions. ARS also used DNA-based methods to characterize the relationships among citrus, coffee and grapevine strains of this pathogen. Lastly through a combination of horticultural, microbiological and biochemical methods ARS has demonstrated that *X. fastidiosa* extensively colonizes sweet orange trees, including the roots, fruit and seeds.

Transmission of *X. fastidiosa* through seed has also been demonstrated for the first time, as has the surprising ability of the citrus strains of *X. fastidiosa* to induce symptoms of Pierce's disease in Chardonay grapevines from California.

Impact: Characterization of strains of *X. fastidiosa* has contributed to changes in bacterial nomenclature of the species, and an appreciation for the role of this organism in natural ecosystems. The labeled strains have demonstrated the pathogen changes expression of genes while *in planta*, possibly while in a biofilm. This work will lead to an improved understanding of how the pathogen is able to move in diseased trees. The extensive colonization of sweet orange trees, fruit and seed that has been documented is potentially of regulatory significance. Improved diagnostic reagents protect domestic sweet orange from foreign strains of *X. fastidiosa*. Reducing losses of citrus by control of plant diseases is the long term objective.

Additional Information: The project has benefited from extensive collaborations with citrus industry scientists at Fundecitrus, São Paulo, Brazil and with scientists at the University of São Paulo. Collaborations have included jointly planned, funded and implemented research projects and scientific exchanges. The collaboration has benefited the United States by allowing our laboratory to have access to bacterial cultures and infected plant material, as well as access to orchards, scientists and laboratories in São Paulo. These interactions are essential for the project, since the pathogen does not occur in the United States.

Documentation:

Qin, X., Miranda, V.S. , Machado, M., Lemos, E. and Hartung, J.S. 2001. An evaluation of the Genetic Diversity of *Xylella fastidiosa* isolated from Diseased Citrus and Coffee. Phytopathology 91(6):599-605.

Li, W., Zhou, C., Pria, Jr., W.D., Teixera, D.C., Miranda, V.S., Pereira, A.J., He, C.-X., Costa, P.I. and Hartung, J.S. 2002. Citrus and coffee strains of *Xylella fastidiosa* induce Pierce's disease of grapevine. Plant Disease 86:1206-1210

Chen, J., Hartung, J.S., Chang, C.J., Vidaver, A.K. 2002. An evolutionary perspective of Pierce's disease of grapevine, citrus variegated chlorosis, and mulberry leaf scorch diseases. Current Microbiology 45(6)423-428.

W.-B Li, W. D. Pria, Jr., P. M. Lacava, J. S. Hartung. 2003. Presence of *Xylella fastidiosa* in Sweet Orange Fruit and Seeds and its Transmission to Seedlings. Phytopathology 93 (8):953-958.

Qin, X. and Hartung, J.S. 2004. Expression of Green fluorescent Protein in *Xylella fastidiosa* is Affected by Passage Through Plants. Current Microbiology 49 (3): 211-216.

Ornamental Viruses

Background: Many plant virus diseases cause significant losses in the production and quality of ornamental crops, are very difficult to control, and new diseases occur as different crops are introduced or grown in new areas. Many crops are susceptible to multiple viruses, each of which may cause serious economic losses, and infected plant material may not be acceptable for sale or export. The primary focus of our research is on those "new" currently uncharacterized or emerging viruses affecting key ornamental crops recently identified as significant to the floral and nursery industry. An understanding of the identity of new and emerging ornamental viruses, as well as their mechanisms of infection, transmission, and pathogenicity, is needed in order to develop better methods of disease control. In crops where no effective resistance to virus infection is recognized, or is available only in distantly related germplasm that is not horticulturally adapted, development of resistance by transgenic expression of portions of the viral genome or antiviral proteins may be the only effective means available. This research is needed to allow increases in both productivity and quality of ornamental plants in an environmentally friendly manner, thereby reducing the use of and reliance on chemical control of pests and diseases.

Accomplishment: Diseases caused by viruses seriously affect the production and quality of ornamental plants. Growers have reported problems with previously unreported viruses in several economically important ornamental crop species exhibiting virus-like symptoms. Using serological and molecular technologies we have determined the identity and performed the initial characterization of several of these new and emerging viruses, and have produced reagents and tools for their detection and diagnosis. A pea mosaic strain of Bean yellow mosaic potyvirus was identified for the first time by serology, cloning and sequencing; this virus was found in a mixed infection with Broad bean wilt fabavirus causing a mosaic disease in *Verbena*. Two new potyviruses infecting the orchid *Spiranthes* have been discovered and portions of their genomes were sequenced. Two additional potyviruses, one associated with flower break symptoms in New Guinea Impatiens and the other associated with leaf mosaic in *Omphalodes*, have been discovered, partially characterized and their 3' terminal genomes sequenced. The complete genomes of four unique viruses infecting geranium, including Pelargonium line pattern virus, Pelargonium ringspot virus, Elderberry latent virus, and Pelargonium chlorotic ring pattern virus, were determined and nucleic acid probes developed for virus detection. Single-chain antibody transgene constructs from broad-spectrum reacting potyvirus monoclonal antibody have been made and used to transform model plants to test the expression of antiviral antibodies in transgenic plants as a novel means of virus control. Other unknown or previously undescribed emerging viruses causing diseases in *Amorphophallus, Bacopa, Lachenalia, Phlox, Scaveola, Tricyrtis*, and *Viola* are currently under investigation. Identification of these new and emerging viruses and availability of reagents for detection will allow growers to test propagation stock in order to select healthy plants, resulting in increased productivity and quality, and customer satisfaction.

Impact: Research to develop tools, reagents and knowledge that will aid U.S. floriculture companies in establishing effective virus testing protocols that will improve clean stock production for new vegetatively-propagated annuals and perennials is an ongoing objective. Other than natural or engineered resistance (which is rare), the

prevention of disease through the development of more effective means for the detection and identification of plant virus diseases affecting ornamentals, and utilization of those methods to allow selection of pathogen-free or pathogen-indexed plants is the best method of controlling viral and bacterial diseases. Examination and characterization of previously unreported viruses causing disease in various ornamental crops has lead to development of diagnostic reagents. The identification of these new and emerging viruses and the availability of reagents for detection will allow growers to test propagation stock in order to select healthy plants, resulting in increased productivity and quality, and customer satisfaction. Improved diagnostic reagents will also help protect domestic crops from existing and foreign pathogens. Reducing losses to crops by control of plant virus diseases is the long term objective.

Additional Information: Three Specific Cooperative Agreements funded through the Floral and Nursery Research Initiative support research on additional viruses of interest to the floral industry. In addition to these three SCAs, further temporary funding from the Floral and Nursery Research Initiative supports work in-house on newly emerging viruses of significance to the floral industry (viruses affecting *Bacopa*, *Scaevola*, and *Verbena*).

Documentation:

Hammond, J. and Jordan, R.L. 2001. *Potyviruses*. in: Encyclopedia of Plant Pathology. John Wiley & Sons, New York. pp. 792-800.

Jordan, R.L, Guaragna, M.A, Kinard, G.R., Lynn, S. 2002. Detection and first report of *Dasheen mosaic virus* and a second potyvirus infecting the terrestrial orchid *Spiranthes cernua.* Acta Horticulturae. v.568 p.253-260.

Jordan, R.L. and Guaragna, M.A. 2002. Successful development of monoclonal antibodies to three carnation viruses, using an admixture of only partially purified virus preparations as immunogen, and their use in virus diagnosis. Acta Horticulture v.568 p.177-184.

Kinard, G.R., Jordan, R.L. 2002. Genome organization of *Pelargonium chlorotic ring pattern virus*: Further implications for Tombusviridae taxonomy. Acta Horticulturae. v.568 p.17-27.

Maroon-Lango, C.J.M., Guaragna, M.A., Jordan, R.L., Hammond, J., Bandla, M., Marquardt, S. 2005. Two unique US isolates of *Pepino mosaic virus* from a limited source of pooled tomato tissue are distinct from a third (European-like) US isolate. Archives of Virology. v.150, in press.

Xyllella II

Background: Bacterial diseases caused, for example, by *Pseudomonas*, *Xanthomonas*, *Xylella*, and *Ralstonia* spp. often result in significant losses in the production and quality

of ornamental crops, and are very difficult to control. Although *X. fastidiosa* has been found to cause leaf scorch diseases of woody ornamentals for 20 years, some of the basic questions still remain largely unanswered. These include what is the host range of *X. fastidiosa* in horticultural and alternative plants, and what are the genetic and pathogenic relationships among strains of *X. fastidiosa* isolated from economically important horticultural and agricultural food hosts and from invasive alternative hosts in the environment. Recent surveys indicate that the disease is spreading and becoming more severe in a number of important landscape trees such as oak, elm and sycamore in many states of the United States. Bacterial leaf scorch of oleander, a relatively new disease that was reported in 1999, is also an emerging problem in California, Arizona, and Texas where oleander is used as a popular landscape plant for residential hedges, highway dividers, colorful accents, and beach plantings. In order to effectively control bacterial leaf scorch diseases of landscape trees and shrubs, answers to the above questions are greatly needed.

Accomplishment: ARS has demonstrated for the first time the association of *Xylella fastidiosa* with disorders of three hosts, including the first report of bacterial leaf scorch in a bonsai tree. ARS also showed the association of *X. fastidiosa* with a leaf-scorch disorder in Japanese beech bonsai and in black oak in the United States, as well as the causal role of *X. fastidiosa* in oleander leaf scorch and the presence of the disease in various locations in Texas. In order to determine how *X. fastidiosa* strains from alternative hosts are related genetically to other hosts, ARS first isolated *X. fastidiosa* from its alternative hosts porcelain berry and wild grape. ARS then determined their genetic relationships with each other and with strains from grape, peach, plum, oak, mulberry, maple and oleander, and found, for the first time, that these alternative host strains of *X. fastidiosa* are more closely related to the oak strain.

Impact: The ARS finding that *X. fastidiosa* is associated with high-valued bonsai is significant because it is an important step leading to the control of the disorder and preservation of the horticultural masterpiece. It is also of great value for regulatory officials when bonsai plants are moved between countries, particularly from the United States to Asian countries where *X. fastidiosa* has not been found. The findings that *X. fastidiosa* is associated with black oak and causes oleander leaf scorch in Texas expand the host range of the bacterium in economically important landscape tree species, and extend the geographic range of this important bacterial disease, respectively. The phylogenetic study revealed for the first time that porcelain berry and wild grape strains are more closely related to the oak strain than to the grape strain, suggesting that they may play an important role in the spread of *X. fastidiosa* affecting economically important hosts such as oak by serving as a reservoir of inoculum in nature. Removal of these alternative host plants in the vicinity of susceptible economic hosts such as oak may be important for the control of the disease.

Additional Information: Two Specific Cooperative Agreements (SCA) funded through the Floral and Nursery Research Initiative support research on *Ralstonia solanacearum.* Under an SCA with the University of Wisconsin-Madison, we determined that symptomless geranium plants infected with *Ralstonia solanacearum* race 3 biovar 2

(R3bv2) release easily detectable numbers of bacteria from their roots; we are working to develop this discovery into a rapid field screening method. The beneficiaries of this research are the geranium production industry, the hundreds of U.S. greenhouse ornamental growers that grow and sell geraniums, and USDA-APHIS-PPQ, the regulatory agency responsible for developing policies that exclude and if necessary, eradicate this Select Agent pathogen. In the second SCA with the University of Florida, research examining bacterial wilt host relationships, and using *Ralstonia solanacearum* Race 1 as a model system, bactericides were screened for their ability to protect geranium plants from infection. Even though strict sanitation procedures have been implemented in geranium production facilities, a low cost effective protectant would greatly benefit the industry. This research finding will provide a direct benefit to the $300 million/year geranium industry and will help safeguard the $1.2 billion/year U.S. potato industry.

Documentation:

Huang, Q., Li, W. and Hartung, J. S. 2003. Association of *Xylella fastidiosa* with leaf scorch in Japanese beech bonsai. Canadian Journal of Plant Pathology 25: 401-405.

Huang, Q. and Sherald, J. L. 2004. Isolation and phylogenetic analysis of *Xylella fastidiosa* from its invasive alternative host, porcelain berry. Current Microbiology 48: 73-76.

Huang, Q. 2004. First report of *Xylella fastidiosa* associated with leaf scorch in black oak in Washington, D. C. Plant Disease 88: 224.

Huang, Q., Brlansky, R. H., Barnes, L., Li, W. and Hartung, J. S. 2004. First report of oleander leaf scorch caused by *Xylella fastidiosa* in Texas. Plant Disease 88: 1049.

Sweet Potato Viruses

Background: Sweet potato virus disease (SPVD) is among the most serious diseases of sweet potato worldwide. Previously, it was thought to be caused by a synergistic interaction of two viruses, sweet potato feathery mottle virus (SPFMV), a potyvirus, and sweet potato chlorotic stunt virus (SPCSV), a crinivirus. However, the etiology of the disease is poorly understood and considerable variation has been reported in the symptoms and severity. Part of the variation in the disease has been attributed to the occurrence of two strains of the chlorotic stunt virus. In order to find effective control, elimination, or prevention methods for the disease, a better understanding of the disease etiology is needed.

Accomplishment: The ARS scientist showed that SPVD could be caused by a synergistic interaction of the SPCSV with any one of several different potyviruses that infect sweet potatoes, not just SPFMV. Furthermore, the research showed that the severity of the disease symptoms varied with the individual potyvirus component of the mixed infection. This was the first report of potyviruses, other than strains of SPFMV, interacting with SPCSV to cause the SPVD.

Impact: The SPCSV is not known to occur in commercial productions of sweet potato in the United States. However, three potyviruses are now known to be present. All three of the viruses were shown to interact with SPCSV to produce the SPVD. One of these viruses, sweet potato vein mosaic virus, was shown to cause an even more severe form of SPVD than produced by the classical SPFMV/SPCSV mixed infection. Therefore, if the SPCSV were introduced into the United States in infected germplasm, the economic impact to sweet potato production could be serious. This new knowledge will be valuable to breeders who are developing new varieties with resistance to SPVD.

Documentation:

Lotrakul, P., Valverde, R., Clark, C., Hurtt, S., Hoy, M. 2002. Sweet potato leaf curl virus and related geminiviruses in sweet potato. Acta Horticulturae 583: 135-141.

Cereal Rusts

Background: The cereal rust fungi *Puccinia graminis* (stem rust of wheat, barley, and oat), *P. triticina* **(**leaf rust of wheat) and *P. coronata* (crown rust of oat) are widespread pathogens of cereal crops throughout the United States and worldwide. The rust fungi are highly variable, as many different physiologic races exist with the ability to cause disease on host cultivars with different combinations of resistance genes. Stable rust resistance in cereal crops has been difficult to achieve due to the high variation for virulence present in rust populations. Genetic characterization of cereal rust fungi, and identification of rust resistance genes in cereals can assist in the development of crop cultivars with durable resistance.

Accomplishment: Collections of leaf rust from throughout the wheat growing regions of the United States were analyzed to identify the most common races present in the United States. Forty-sixty different races were identified annually on a set of 16 near-isogenic lines of Thatcher wheat that differ for single leaf rust resistance genes. Races with virulence to *Lr9, Lr11,* and *Lr18* are common in the soft red winter wheat region of the southeastern states; races with virulence to *Lr17,* and *Lr41* are common in the hard red winter wheats of the southern Great Plains; and races with virulence to *Lr2a* and *Lr16* are common in the spring wheat area of the northern Great Plains. Races of leaf rust with high virulence to durum wheat from Mexico, South America, Europe and California were characterized for virulence to the Thatcher lines. The durum leaf rust races were very similar or identical for virulence to the Thatcher lines and were virulent to all durum cultivars tested from the United States, Canada, and CIMMYT.

Impact: The rust fungi that attack small grain cereal crops are highly variable and dynamic pathogens that often overcome disease resistance genes in cereals. Proper identification and characterization of rusts that attack wheat, oats, and barley, will enable plant pathologists and plant breeders to select crop genotypes with combinations of resistance genes that will provide more durable resistance to the rust pathogens. Identification of rust resistance genes that provide durable resistance and molecular

markers associated with these genes will assist in the development of crop cultivars with durable resistance. This research will result in reduced crop losses caused by cereal rust diseases.

Documentation:

Leonard, K.J., Anikster, Y., and Manisterski, J. 2004. Patterns of virulence in natural populations of *Puccinia coronata* on wild oat in Israel and in agricultural populations on cultivated oat in the United States. Phytopathology 94:505-514.

Anikster, Y., Szabo, L. J., Eilam, T., Manisterski, J., Koike, S. T., and Bushnell, W. R. 2004. Morphology, life cycle biology and DNA sequence analysis of rust fungi on garlic and chives from California. Phytopathology 94: 569-577.

Kolmer, J. A., Long, D. L., and Hughes, M. E. 2004. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2002. Plant Dis. 88: 1079-1084.

Biochemical Processes in Nematodes

Background: Plant-parasitic nematodes are serious pathogens attacking all agricultural plant species, and cause nearly 10 billion dollars in crop losses annually in the United States. Nematode control has usually relied upon the use of highly toxic and expensive chemical pesticides, several of which have been withdrawn from use because of environmental concerns, and the continuous development and introduction of resistant cultivars, which are not always effective. Therefore, there exists an urgent need for environmentally safe, cost-effective means of control. The research program identifies endogenous mechanisms that regulate fundamental processes, including development, reproduction, metabolism, and response to stress, and characterizes the molecules and genes involved. The overall goal is to disrupt nematode growth and development through the exploitation of specific molecular targets. Results of this research will facilitate the development of selective control methods based upon nematode-specific biology.

Accomplishments: *Hsp90 molecular genetics and physiological function*. Hsp90 proteins are ATP-binding molecular chaperones that are needed to protect key regulatory proteins from improper folding, especially during times of stress. ARS was the first to isolate an Hsp90 gene from any plant-parasitic nematode, and the first to demonstrate that Hsp90 from the soybean cyst nematode (*Heterodera glycines*) forms functional cross-species dimers with Hsp90 from the free-living nematode *Caenorhabditis elegans*. ARS also recognized the value of geldanamycin, a natural product and a specific inhibitor of Hsp90, for the study of plant-parasitic nematode egg hatching and motility. Using the soybean cyst nematode, *Heterodera glycines*, and the free-living nematode, *C. elegans*, ARS was the first to successfully demonstrate the adverse effects of geldanamycin in any nematode species.

Endogenous neuropeptides and proteases in development. The FMRFamide family of neuropeptides regulates muscular activity in invertebrates, and we discovered

biochemical differences among these neuropeptides at different developmental stages of H. *glycines*, and between *H. glycines* and free-living nematodes. ARS also discovered proteases in *H. glycines* that can regulate neuropeptides, including an aminopeptidase that we characterized, determined its sub-cellular distribution, compared it with an analogous *C. elegans* enzyme, and proposed its role in neuropeptide metabolism in *H. glycines*. ARS also discovered a proprotein processing enzyme gene in *H. glycines*, the first for any parasitic nematode, and characterized a gene coding for a protease that may have a role in hatching.

Sterol and glycosphingolipid biochemistry in nematodes. Surprising differences exist between nematodes and plants with respect to glycosphingolipid and sterol biochemistry. Although previous Nematology Laboratory research indicated that root-knot nematodes possess large quantities of simple glycosphingolipids with an unusual sphingoid moiety not found in plants, ARS has recently discovered that the compounds do not occur in the soybean cyst nematode. ARS has also been the first to use an inhibitor to specifically disrupt the sterol 4-methylation pathway in *C. elegans*; this pathway is unique to nematodes. Additionally, ARS was the first to discover that specific sterols in *C. elegans* can enhance or decrease the resistance of nematodes to various stresses.

Impact: *Hsp90 and geldanamycin.* The Hsp90 research is a critical step towards unraveling the neurosensory pathway that determines how internal or external signals influence development and lifespan in plant-parasitic nematodes. By correcting misconceptions about the use of geldanamycin to disrupt Hsp90 function, and demonstrating its effect in nematodes, others have begun to use geldanamycin in novel research. These discoveries indicate that chemical, microbial, or bioengineered inhibitors of Hsp90 may form the basis for the development of safer, biologically based nematode control strategies.

Endogenous neuropeptides and proteases. Characterization of aminopeptidase activity and the discovery of a processing enzyme gene in *H. glycines* will provide the basis to develop a model for neuropeptide regulation in this nematode. Biochemical differences discovered between *H. glycines* and free-living nematodes, including *C. elegans*, and discovery of a protease gene with potential involvement in hatching, suggest that we or other scientists will be able to develop a highly specific control agent.

Sterol and glycosphingolipid biochemistry. The difference between root-knot and cyst nematode glycosphingolipid biochemistry is one of the few fundamental biochemical differences between the major two groups of plant-parasitic nematodes. Further investigation could lead to exploitation of these differences by novel control strategies. Discoveries about the roles of specific sterols in nematodes and inhibitors that disrupt their biosynthesis will be used by us and others for developing new methods of controlling the damage caused by plant-parasitic nematodes.

Additional Information: Significant collaborations and sources of funding include: All-Russian Research Institute of Phytopathology, Golitsino (financial support for collaboration from International Science and Technology Center); University of

Medicine and Dentistry of New Jersey, Piscataway, New Jersey; Department of Crop Science, University of Illinois, Urbana, Illinois; Yonsei University, Seoul, Korea; University of Maryland, College Park Maryland; Donald Danforth Plant Science Center; United Soybean Board, Application of Biotechnology to Control the Soybean Cyst Nematode.

Documentation:

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Sorghum Microbe Interactions

Background: The overall goal of the CRIS is genetic improvement of sorghum for enhancing energy and nutrient availability, as well as disease resistance. Therefore, much of the sorghum being utilized in this research has had modifications in secondary metabolism, which was accomplished genetically. Since many disease resistance responses are products of secondary metabolism or are induced by a product of secondary metabolism, modifications to these pathways can directly impact interactions with microorganisms. For example fungal or bacterial strains that are relatively innocuous, may be capable of causing disease in plants that have been impaired in resistance responses. On the other hand, if perturbations in secondary metabolism results in the build-up of compounds potentially toxic to microorganisms, the cultivar may be more resistant to at least one or a few potentially pathogenic strains. Additionally, variations in secondary metabolites may affect growth of mycotoxigenic fungi following harvest and in long-term storage, a potentially serious problem for livestock and human consumers.

Accomplishments: Previously described protocols, or modified and improved previously described protocols, and new protocols were developed by ARS to isolate, identify and assess pathogenic, cultural and genetic differences between fungi and bacteria associated with sorghum. These techniques are being used to assess species and pathovar differences of fungi isolated from near isogenic cultivars varying in plant and seed pigments or reduced in lignin content. ARS also assessed the impact of the sorghum allelopathic compound, sorgoleone, on soil microbial communities, including pathogens, saprophytic microorganisms potentially antagonistic to pathogens and on other plants that may be in rotation with high-sorgoleone producing sorghum cultivars.

Impact: The project has yielded genetic material that will possibly be used to generate commercial lines lowered in lignin for the ethanol industry or increased nutrients for livestock, or lacking pigments that effect taste and quality for livestock or even human consumption. It is essential that information exist regarding the potential for increased disease resistance or susceptibility in these genetic lines. Research assessing the impact of sorghum root-produced toxins on soil microorganisms and on other crop plants, will be informative for cropping systems involving sorghum. Presentation of research on plant and seed color and on sorgoleone was well received, especially by extension researchers.

Additional Information: MTA through which experimental germplasm lines were provided to Penn. State University: Infection of sorghum with the fungus *Cochliobolus heterostrophus* stimulates the synthesis of 3-deoxyanthocyanidins that act as phytoalexins. Differentially expressed cDNA corresponding to a *flavonoid 3'-hydoxylase* gene was shown to be coordinately regulated transcriptionally and expression of these genes was induced within the first 24 hours of fungal infection. The order of synthesis of apigeninidin and luteolinidin shows a coordinated response with the temporally induced expression of the *f3'h* gene. This information adds to the understanding of how plants respond to pathogens at the molecular level and will aid in developing systems to combat plant diseases.

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SCA 58-5440-1-329 and SCA 58-5440-8-134 funded collaborative pathology research the University of Nebraska: A DNA based method was developed allowing *Claviceps africana* and *Claviceps sorghicola* to be identified to species in the presence of other sorghum-associated organisms without the need for culturing. It was determined that *Claviceps africana* honeydew protects macroconidia from both high and low temperature extremes providing a survival mechanism for the disease. It was demonstrated that in four sorghum hybrids, no differences existed between A1 and A2 cytoplasms for susceptibility to head blight and grain mold, indicating that use of A2 cytoplasm to increase genetic diversity of grain sorghum hybrids, should not increase risk due to these two diseases.

Documentation:

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Fireblight

Background: Comprehensive knowledge of the biology of microorganisms associated with tree fruits, including pathogen taxonomy, disease etiology, and disease detection is lacking. Such knowledge is critical for development of science-based phytosanitary regulations and mitigative measures for imported and exported tree fruits. Mis-identification of fungal and bacterial pathogens of tree fruits can lead to the importation of exotic pathogens and diseases, and can hinder progress in development of export markets for U.S. commodities. To address these issues we are studying pathogen biology of selected agents, including *Alternaria* spp. (causing several different fruit diseases) and *Erwinia amylovora* (causal agent of fire blight disease) and are conducting morphotaxonomic and DNA fingerprinting studies necessary to develop DNA-based methods to detect and identify exotic and domestic fruit pathogens in the genus *Alternaria*.

Accomplishment: Since it began in 1995, participation in the U.S. apple export program to Japan decreased each year until 2001, when no U.S. apples were exported to Japan. The program requirements were overly restrictive and expensive for growers, and were not supported by the available scientific evidence or a pest risk assessment for dissemination of fire blight disease by commercial apple fruit published in 1998. From March through December 2000, ARS hosted and conducted joint U.S.-Japan field experiments to determine if the 500-meter buffer zone and three inspections required by Japan were scientifically justified. ARS negotiated the experimental protocols with Japan-MAFF (government agricultural agency), and supervised the subsequent field and laboratory experiments. The experiments confirmed previously published results; no *E. amylovora* was detected inside mature apple fruit, even when harvested from severely blighted trees. Additionally, no fire blight symptoms developed in any of more than 30,000 fruit harvested and stored until December 2000.

Impact: Despite these results, Japan refused to amend their regulations governing importation of U.S. apple fruit. Based upon the results of the joint U.S.-Japan experiments and earlier publications, the U.S. government initiated a WTO dispute resolution action through the Office of the U.S. Trade Representative. The first panel convened in Geneva in October 2002, and an Expert's session was convened in March 2003. The outcome of this was a very favorable ruling by the WTO panel. Japan appealed the ruling to the WTO Appellate Body, and lost. After several Implementation meetings during 2004 with Japan MAFF to establish a new apple export work plan consistent with the WTO ruling, Japan allowed the June 30, 2004, deadline to lapse and moved into default. In July 2004, the U.S. government initiated a non-compliance action against Japan. The WTO Panel heard arguments on the non-compliance issue from the United States and Japan and reconvened the Expert Panel in October 2004. During these proceedings, the Experts confirmed the lack of a factual basis for buffer zones of any size and the lack of evidence to support even one field inspection for fire blight. The non-compliance ruling will be issued in mid-March 2005. If Japan continues to maintain its regulations without scientific basis, the United States has initiated a request through the WTO to assess more than $143.4 million dollars in compensatory tariffs each year against imported Japanese goods (the U.S. estimate of the yearly value of lost trade). This entire WTO action will result in expansion of grower opportunities to access the Japan market (and others such as Australia, China, South Africa) and to increase profitability for our growers.

Additional Information: Funds were obtained from the Washington State Tree Fruit Research Commission and by Japan-MAFF.

Documentation:

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Andersen, B.A., Kroger, E. Roberts, R.G. Chemical and morphological segregation of *Alternaria Arborescens, A. infectoria* and *A. tenuissima* species-groups. Mycological Research. 2002. V.106(2). P. 170-182.

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Roberts, R.G. *Alternaris yalinficiens sp.Nov on Ya Li pear fruit: from interception to identification.* Plant Disease. 2005. V. 89. P. 134-145.

Wheat Virus

Background: Current strategies for reducing incidence of Wheat streak mosaic virus (WSMV) in winter wheat are limited to cultural control methods. WSMV is one of the most economically important viral disease agents of wheat in the United States. The overall goal is to relate wheat virus gene structure to function and to assess parameters that shape wheat virus population structure. Improved understanding of the genetic basis of viral pathogenesis, vector transmission, recombination, and population genetics will facilitate efforts to ameliorate the effects of viral infection in wheat and other cereal crops. Presently, available resistance to wheat viruses is limited and inadequate. Detailed knowledge of wheat virus gene function affecting important pathogenic properties and transmission will provide a rational basis to identify specific components of the viral life cycle that may be exploited to develop novel control strategies. Knowledge of wheat virus diversity, population genetics, and capacity to change will define how robust a durable control strategy will need to be. It is likely that the knowledge obtained in this research will be directly applicable to a wide range of virus-host plant combinations.

Accomplishments: Full-length cDNA clones of WSMV have been constructed and used to generate infectious viral RNA that may be inoculated to plants, permitting analysis of WSMV gene function using a 'reverse genetics' approach. Using this approach ARS has determined that the WSMV P1 gene functions as a serine proteinase required for polyprotein processing during expression of the WSMV genome. Mutational analysis of the WSMV HC-Pro gene has indicated that this multi-functional protein is required for virus transmission by eriophyid mites, long distance systemic movement within plants, polyprotein processing (via cysteine proteinase activity), and serves as a determinant of host range and virulence. Mutational analysis of the WSMV P3 gene yielded a surprising result: mutations in the viral RNA that did not alter the encoded P3 protein nonetheless resulted in loss of systemic infection in wheat. This observation suggests that the P3 gene also contains a cis-acting RNA element required for infectivity and explains why the P3 gene is highly conserved among WSMV strains. ARS has successfully inserted reporter genes into the WSMV infectious clone, demonstrating that WSMV may be used as a gene expression vector to introduce foreign genes into wheat. This WSMV gene expression vector may have utility for use in functional genomics of wheat and other cereals. The complete nucleotide sequences of five WSMV strains and three other viruses (Oat necrotic mottle virus, Agropyron mosaic virus, and Hordeum mosaic virus) related to WSMV have been determined. These sequences have been deposited in a public database (GenBank) and used by us to clarify genetic relationships and biogeography of eriophyid mite transmitted viruses of wheat and other cereals. ARS has also determined (and deposited) coat protein gene sequences of ~50 additional isolates of WSMV. These data allowed ARS to examine the population structure of WSMV in the United States. Population genetics studies have indicated that WSMV (and presumably all plant viruses) are subjected to severe bottleneck events during systemic movement and vector transmission. ARS has developed a model for plant virus population genetics in which both random events (e.g. genetic drift) and selection operate concurrently to shape virus populations and their evolution. These results directly conflict with the prevailing

view of virus population genetics, and indicate that the currently accepted model of virus population genetics is not applicable to WSMV and, most likely, all plant viruses.

Impact: The project developed significant new information on WSMV gene structure and function with respect to pathogenicity and virulence. This information is directly applicable not only to wheat streak mosaic disease, but also to the numerous viral diseases of other crops caused by potyviruses. Information gained by reverse genetics may be exploited to generate dominant negative gene constructs in which mutated forms of viral proteins expressed in plants could result in improved plant resistance to viruses. Studies demonstrating that the HC-Pro gene of WSMV is required for vector transmissibility provided the first molecular and mechanistic information on transmission of plant viruses by eriophyid mites. Sequences deposited in GenBank provide other scientists with information needed to develop nucleic acid-based diagnostics for cereal viruses and serve as references to define relationships with other viruses. The WSMV gene expression vector was the first such construct for cereals and may facilitate functional genomics of wheat. Knowledge of wheat virus diversity, population genetics, and evolution may aid in prediction of resistance durability.

Additional Information: Informal collaborative efforts have been established with non-ARS scientists. ARS has interacted extensively with University of Nebraska Professors. This collaboration has worked well in our efforts to understand fundamental aspects of vector transmission of WSMV. The University of Nebraska has provided the wheat curl mite colonies and facilities necessary to conduct vector transmission assays. The most recent result of this collaboration is the finding that the WSMV HC-Pro gene is a viral encoded genetic determinant required for virus transmission by eriophyid mites. These studies have defined the population structure of WSMV in the United States and clearly demonstrated that unique genotypes of WSMV are present in Europe and Mexico. This research has not been of a formal nature nor have they involved transfer of internal or external funds.

Documentation:

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Septoria Leaf Spot

Background: Septoria leaf spots are economically important fungal diseases occurring in many small grain crops and common grasses. The diseases are epidemic world-wide and cause yield loss especially during wet growing seasons. Septoria leaf spots of cereal is a disease complex caused by a number of fungi that are not always closely related. *Septoria* species important on cereals are placed in the Loculoascomycetes, with known teleomorphs in *Mycosphaerella* (anamorphs *Septoria*) and *Phaeosphaeria* (anamorphs *Stagonospora*). The four fungal pathogens of the *Septoria* group that have had the greatest impact on overall agriculture in cereals are *Septoria tritici*, *Septoria passerinii*, *Phaeosphaeria nodorum* and *Phaeosphaeria avenaria*. Cereal *Phaeosphaeria* pathogens have traditionally been distinguished by pycnidiospore morphology and host pathogenicity. *Phaeosphaeria avenaria* includes two *formae specialis*, *P. a.* f. sp. *avenaria* (Paa) and *P. a.* f. sp. *triticea* (Pat). Paa incites a leaf disease on oat and Pat has broad host range infecting wheat, barley, rye and several common grasses. Since pycnidiospore size varies within single-spore cultures derived from the same isolate and is influenced by environmental conditions such as culture media and temperature, cereal *Phaeosphaeria* species identification is complicated and difficult in certain occasion. On the other hand, genetic diversity can be found in various *Phaeosphaeria* species with similar pycnidiospore morphology. Pathogens may have different levels of sensitivity toward fungicides applied and exhibit different degree of virulence to various host plants. If the pathogens are incorrectly identified, the control strategies currently in use may not be effective.

Application of molecular approaches to study phylogenetic relationships in *Phaeosphaeria* and other ascomycetes, by sequence analyses of mitochondrial rDNA and mating type conserved regions, genes encoding enzymes related to cell metabolism and growth, and those expressed during fungal infection may facilitate the identification of *Septoria* complex in cereal leaf spots diseases. Development of a more reliable method for *Septoria/Stagonospora* species identification will help to diagnose cereal *Septoria* diseases and to establish the strategy for potential disease control. The results will also facilitate the development of a phylogenetic hypothesis for the deep branches within the Kingdom of Fungi and enhance research and educational tools in fungal systematics.

Accomplishment: Genetic relatedness based on mating type gene (*MAT1*) conserved region sequence and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene in two cereal *Phaeosphaeria* species, *P. nodorum* and *P. avenaria*, corresponds to the results previously found by the restriction fragment length polymorphism (RFLP) fingerprinting

and ribosomal internal transcribed spacer (ITS) region sequence analysis. The glyceraldehyde-3-phosphate dehydrogenase protein sequence in two cereal *Phaeosphaeria* species was identical and is closely related to the protein of maize fungal pathogen, *Cochliobolus heterostrophus*. The molecular methods developed can be used to assist and complement the traditional fungal classification and identification. Discovery of sole *MAT1-1* gene in homothallic *P. avenaria* f. sp. *triticea* (Pat1) and sub grouping of *P. nodorum* based on *gpd* gene intron region provide new knowledge in fungal genetics and evolution for scientific community.

Impact: DNA probes and gene clones from *Phaeosphaeria* pathogens have been distributed to scientists for research purposes. Molecular identification through DNA amplification and enzyme restriction is developed for various cereal *Phaeosphaeria* pathogens. Improved diagnostic reagents will help protect domestic crops from existing and foreign pathogens.

Additional Information: In collaboration between the Plant Breeding and Acclimatization Institute, Poland, and the Molecular Plant Pathology Lab, USDA-ARS, Beltsville MD, we identified quantitative trait loci (QTL) associated with Stagonospora nodorum blotch disease resistance (SNB) in a partially resistant wheat cultivar 'Alba' by using pollination and tissue regeneration to produce double haploid plants. Using microsatellite DNA amplification, two genetic markers associated with SNB resistance were identified. These two markers on chromosomes 6A and 6D could be transferred into various genetic backgrounds to elevate wheat resistance to SNB.

Documentation:
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Cercospora Fungi

Background: The filamentous fungi belonging to the genus *Cercospora* comprise a large group of agriculturally-destructive pathogens. Members of this genus are well known for their production of secondary metabolites, many of which are phytotoxins. The light-activated perylenequinone toxin, cercosporin, has been the focus of much study due to its importance in plant disease, its unusual photochemistry, and its general toxicity to a wide range of living cells. For the species of this genus infecting sugarbeet, *C. beticola*, additional toxins termed the beticolins have also been implicated in disease induction. Mutagenesis studies combined with gene transfer have demonstrated the importance of cercosporin in the infection of soybean by *C. kikuchi.* Thus, a collection of chemically-induced mutants deficient in cercosporin production were shown to be unable to infect healthy soybean plants. Likewise, a gene product from *C. kikuchii* with similarities to multi-drug efflux pumps and required for cercosporin export was found to be necessary for pathogenicity on this host. Although sugarbeet has been used in the characterization of the cellular effects of cercosporin and the beticolins , analogous mutational studies in sugarbeet/*C.beticola* pathosystem or other *Cercospora* pathogens have yet to be done.

Accomplishment: A portion of the CTB gene encoding an enzyme in the cercosporin biosynthetic pathway of *Cercospora* species was cloned from *C. beticola*. Using a transformation vector harboring these sequences, the endogenous CTB gene in *C. beticola* was disrupted by homologous recombination. Southern and northern blot analysis confirmed that transformants lacking secretion of the red pigmented cercosporin toxin possessed a disrupted CTB gene and lacked vector integration events in other regions of the genome. Five CTB mutants (ctb- 2, - 3, - 21, - 23, and - 24) examined produced reduced cercosporin as compared to parent C. beticola isolate 303B. All isolates possessed radial growth rates indistinguishable from 303B and retained the ability to sporulate in culture. Inoculation of sugar beet plants with the five mutants induced a lower number of leaf spot lesions that expanded at a reduced rate as compared to that produced by isolate 303B. Cercosporin accumulation in leaves infected with the mutants as compared to that from leaves infected by the parent is currently being determined. The data indicate that cercosporin is a virulence factor in the infection of sugar beet by *C. beticola*.

Impact: Cercosporin toxicity has been a key focus of damage caused to sugarbeet leaves even without proof that its absence in the fungus results in loss of pathogenicity. This work establishes that cercosporin is a virulence factor, not pathogenicity facton, in the cause of leaf spot disease: Other unknown factors are therefore important in generating disease. Therefore, involved methods for screening or engineering sugarbeet germplasm for cercosporin resistance should be weighed against the real gain that would be achieved in resisting leaf spot disease in the field.

Additional information: Dr. Kuang-Ren Chung of the University of Florida cloned the original CTB1 gene from Cercospora kikuchii. He performed the gene ablations in our reference isolate of C. beticola. The laboratory of the PI has confirmed the ablation of

CTB1 sequences using standard molecular techniques and the reduction in virulence to sugarbeet leaves and the UDSA-ARS, NCSL in Fargo is performing cercosporin analysis on infected leaf samples.

Documentation:

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Pepino Mosaic Virus

Background: Diseases incited by **viruses, bacteria, and fungi cause serious losses to production of vegetable crops.** This new project was established April 25, 2003 to develop effective, biologically-based management practices that do not rely on conventional pesticides to control these diseases. New and effective control measures are greatly needed against viral, bacterial, and fungal diseases, because these diseases of vegetable crops are major production limiting factors. For many vegetable crop diseases there are no known control methods that are both effective and economically feasible. For many other diseases effective current control methods rely on conventional pesticides which are being withdrawn. Biologically-based, more environmentally-compatible strategies are greatly needed to provide effective controls that are economically feasible, environmentally compatible, and acceptable to consumers. Such control methods are critical to ensuring a continued, affordable, safe supply of nutritious vegetable crops.

Accomplishment: This is a new project with two new scientists reported on duty only in May and September 2004 respectively. Nevertheless, ARS has made some good progresses during this short period. Pepino mosaic virus (PepMV), a member of Potexvirus in plant virus, is an emergent virus of tomato. It has only been observed in the United States since 2000. ARS determined the nucleotide sequence of the complete viral genome and have found that there are two distinct isolates mixed infected in the tested plants. This sequence information allowed ARS to develop a rapid and sensitive detection method with Realtime RT-PCR technology.

Impact: Pepino mosaic virus was originally identified in the Andean region of South America. Although several European isolates of this virus have been completely

sequenced, tomato isolates from Peru or Chile have yet to be sequenced. Understanding the viral genome sequence from the center of origin would allow us to evaluate its relationship with other isolates and the evolution of this virus. In additional, the sequence information will allow us to generate resistant tomato plants through pathogen-derived-resistance.

Documentation:

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Discovery Area II: Population Dynamics and Spread, Epidemiology, Forecasting.

Phytophthora

Background: Soybean yield losses due to diseases have increased during the past ten years. Two major diseases, Phytophthora root rot and sudden death syndrome, are currently being investigated. The research emphasizes investigations on the host and pathogen to improve resistance to highly virulent isolates of and extensive yield loss. Identification and maintenance of new and important races or pathotypes of these two soybean pathogens are being developed and used in disease control research that will minimize yield losses and help keep soybean varieties in the Midwestern states resistant to major diseases. Documentation of new races/biotypes of two major soybean pathogens *Phytophthora sojae* races and *Fusarium solani* f. sp. *glycine* that cause root rot and identification of host resistance permit enhancement of soybean germplasm and contributes to the development of soybean cultivars that minimize yield losses causes by soybean disease pathogens. Ten years ago races of the oomycete fungus causing Phytophthora root rot were controlled by growing soybean varieties with the *Rps1-k* gene. The disease, caused by specific races of *Phytophthora sojae*, was first reported in 1951 in Ohio and Indiana and quickly became a major threat to U.S. soybean production. Root infections by *P. sojae* damage soybeans at all growth stages. In seedlings, it causes a rotting called damping-off. Later, it causes the stem and lower branches to turn brown, while leaves turn yellow and wilt, or if conditions are less favorable for the disease, the pathogen may invade roots and reduce yields without showing any visible above ground symptoms. This disease is potentially devastating in soybean varieties that do not have *Rps* genes that confer resistance to specific races of the pathogen. Currently, there are forty-five races reported. Nineteen new races have been described during the past ten years (seven new races reported in Indiana by this on-going project). Information is limited that verifies the race situation in most soybean production areas. However, Indiana and Ohio were found to have the greatest race diversity among the Midwestern

states. Effective control by *Rps* resistance genes requires information about new and dominant pathotypes of *P. sojae*.

Accomplishment: Since several of the new *P. sojae* races can attack soybeans with the *Rps1-k* gene, race verification and population dynamics of this important soybean pathogen were investigated in this research. Races 1, 3 and 4 have consistently been the most commonly isolated races in the Midwest, but additional races have been reported sporadically in the late 1990s. Soil samples from randomly selected Indiana soybean fields and plant samples from selected field nurseries were utilized in the study. Currently, races 1, 3, 4, 7, 25, 28, 33, 43, 44 and 45 are identified as Indiana's most common races. This information documents the importance of determining distribution and frequency of races that can be used to maximize disease control strategies. The *Rps1-k* gene confers resistance to races 1, 3, 4, 7, 43 and 44; however, to control races 25, 28, 33 or 45, the *Rps3* or *Rps8* gene is needed to develop soybeans with improved resistance. These *Rps* genes can be combined with the *Rps1-k* gene to improve resistance and minimize yield losses. Utilization of soybeans with *Rps*3 resistance is particularly important in high-risk production environments until the recently identified *Rps*8 gene is more readily available.

Impact: Since the initiation of *P. sojae* race assessment in the 1990s by similar projects have developed in other states and currently a North Central Regional project is being funded by North Central State Soybean Boards. Yield reductions due to Phytophthora root rot were estimated at $124,000,000 for the United States annually in the 1990s. Fungicide seed treatments are used to reduce seedling damage, but managing *P. sojae* with genetic resistance currently available would result in significant economic benefit and reduce the need for fungicide treatments.

Yield losses due to root rot occur regularly and account for millions of dollars annually. Sources of resistance are not available for many of the root rot diseases, but for Phytophthora root rot, excellent sources of resistance are available. This disease caused by specific races of a soilborne pathogen *(P. sojae*) can be controlled by unique, naturally occurring disease resistance genes. The "*Rps*" or resistance genes trigger the production of an antifungal compound (phytoalexin 'glyceollin') or "natural occurring antibiotic" in soybeans. Production of the glyceollin is initiated when the pathogen infects or attacks the soybean plant. Within 24 hours after infection, production of the antifungal compound protects the plant from the *Phytophthora sojae* pathogen.

The soybean disease research described above is needed to devise effective management strategies to keep up with the changing disease situation. Documentation of new races or biotypes of major soybean pathogens and identification of host resistance permit enhancement of soybean germplasm and contributes to the development of soybean cultivars that minimize yield losses causes by soybean disease pathogens.

Additional information: Research grant funding from the Indiana Soybean Board, North Central Soybean Project, and designated pathogen germplasm funds from the University of Georgia (Soybean Pathogen Germplasm collaborators – USDA IFAFS Grant) have enhanced the soybean disease research. Funds have been used primarily for additional

technical support needed to maintain and verify the *P. sojae* race identification of isolates collected during the long-term study.

Documentation:

Baird, R. E., Abney, T. S., and Mullinix, B. G. 2001. Fungi associated with pods and seeds during the R6 and R8 stages of four soybean cultivars in southwestern Indiana. Canadian Journal Phytoprotection 82:1-11.

Cochran, A. J. and Abney, T. S. 2000. Phytophthora root rot of soybeans: Dominant races and strategies for effective control. Proceedings Midwest Soybean Conference (2000). Indianapolis, IN. pp.43-48.

Dorrance, A.E., Jia, H. and Abney, T.S. 2004. Evaluation of soybean differentials for their interaction with *Phytophthora sojae*. Plant Health Progress. 309(1). Available from: http://www.plantmanagementnetwork.org/sub/php/research/2004/psojae/

Stem Rusts of Grasses

Background: Growers in the Pacific Northwest produce over 90 percent of the U.S. crop of certified perennial ryegrass and tall fescue seed. Combined with orchard grass, bentgrass and fine fescue acreages, grass seed crops occupy over 586,000 acres in the region with a farm gate value in excess of $450 million. This seed provides the basis for grassland agriculture in the United States where much beef and dairy production is dependent on stable supplies of seed for high quality livestock forage. The seed also is exported to global markets for use in pasture, turf, recreational, and reclamation uses. Economic production of this seed and its utility in export markets is dependent in part on effective disease control during seed production and the ability to detect and identify disease organisms in seed lots destined for export markets. Economically, the most damaging disease to the crop is stem rust. Growers spend $10 million annually on fungicides to control the disease, and most apply these fungicides at regular intervals without regard to actual disease development because reliable disease warning information has not been available. A reliable decision aid based on research about factors that impact stem rust development would permit growers to forego one or more fungicide applications per season and make the applications when they will provide the most economic benefit.

Accomplishments: Development of a mathematical model that describes stem rust epidemic development in turf and forage grasses based on measurable weather factors and implemented the model on a publicly-accessible Internet website. The user interface permits inputs of grower observations to model disease development for specific fields. Website operation includes real-time, site-specific weather from automated weather stations in grower fields, and is a collaborative effort with industry and Oregon State University. The model is based on several years of field, greenhouse and laboratory research which we have reported in the scientific literature. ARS developed algorithms based on this research that estimate infection severity from a combined wetness/heat unit

calculation, an approach which has application for many plant diseases. This research provided the first explanation and quantitative description for within-plant disease spread, an important process for stem rust diseases. Measurable effects of planting date adjustment on subsequent disease development were demonstrated and reported, and an important genetic subdivision within the stem rust pathogen population was documented.

Impact: The model is currently being used by seed growers to evaluate the hazard of stem rust disease development in specific fields based on local weather and their own field-specific observations to plan fungicide applications in accordance with an economic threshold specified in the model. Reduction of one fungicide application on an average sized grass seed farm (350 acres) reduces the the input costs for stem rust control by $7000. On a regional basis, elimination of one scheduled spray on half the acreage of perennial ryegrass reduces aggregate pesticide costs by $1.8 million and reduces pesticide use by 12,000 gallons of technical grade product per year. One component of the research included a Memorandum of Understanding which provided disease algorithms to a private-sector manufacturer of automated weather stations for agricultural use. In addition to the benefits provided to growers and industry through technology transfer, the basic research results provided new concepts and methods of botanical epidemiology applicable to other rust diseases and to plant disease epidemiology in general. In particular, this research demonstrated the integration of plant phenology and environmentally-driven infection biology into a predictive model for disease development. This model serves as an example for improving crop disease management methods to reduce pesticide inputs and prevent yield losses.

Documentation:

Pfender, W. F. 2001. Host range differences between populations of *Puccinia graminis* subsp. *graminicola* obtained from perennial ryegrass and tall fescue. Plant Disease. V. 85 p. 993-998.

Pfender, W. F. 2003. Prediction of stem rust infection favorability, by means of degree-hour wetness duration, for perennial ryegrass seed crops. Phytopathology. V. 93 p. 467-477.

Pfender, W. 2004. Role of phenology in host susceptibility and within-plant spread of stem rust during reproductive development of perennial ryegrass. Phytopathology. V. 94 p. 308-316.

Pfender, W. F. 2004. Effect of autumn planting date and stand age on severity of stem rust in seed crops of perennial ryegrass. Plant Disease. V 88:1017-1020.

Karnal Bunt

Background: The Karnal bunt pathogen, *Tilletia indica*, is an obligate outcrosser and depends on encounters on wheat spikes between airborne spores of different mating types for successful infection and reproduction. This life history characteristic results in

reduced reproductive success for lower population densities. Such density-dependence at low population levels has been described for a range of animals and plants and is often termed an Allee effect. The objective was to understand how the Allee effect may affect the epidemic potential of this economically important pathogen.

Accomplishment: ARS developed a simple population model of T. indica that incorporates an Allee effect by calculating the probability of infection for different numbers of spores in the infection court. This work was done in collaboration with Kansas State University, CIMMYT, Mexico City, and Punjab Agricultural University, Ludhiana, India.

Impact: An Allee effect is predicted to be important at the frontier of an invasion, for establishment of new disease foci by a small population of resting spores, and when the environment is nonconducive for the production of new infective spores. Using the model estimates, we demonstrated a theoretical Allee threshold population size below which populations of *T. indica* were predicted to decline rather than increase. The threshold depended on the reproductive rate of the pathogen. Deployment of partial resistance and use of fungicides may be more useful if they push population levels below the Allee threshold. This Allee model has informed efforts by USDA-APHIS to model invasion pathways of this pathogen and has been used to justify reducing international quarantine regulations.

Documentation:

Garrett, K. A. and Bowden, R. L. 2002. An Allee effect reduces the invasive potential of Tilletia indica. [Erratum: 2004 Jan., v. 94, no. 1, p. 120.]. Phytopathology. 92:1152-1159.

Citrus Diseases

Background: Multiple pathogens are responsible for production losses in the United States citrus, vegetable, ornamental and stonefruit industries. New and/or more severe plant diseases may indicate the existence of new plant pathogens or the appearance of more virulent strains of existing pathogens. In both cases, the synthesis of useful tools that diagnose and differentiate pathogens requires the integration of data on the biological, serological and genetic attributes of these pathogens. The successful management of existing disease problems can benefit from new diagnostic tools developed with data generated from the application of new genetic technologies to pathogen identification and differentiation not possible using previous research capabilities. Epidemiological analyses can lead to more effected survey methods and understanding of how diseases spread can lead to more effective eradication strategies. These tools can be applied toward the eradication or management of the pathogen in the field or in source materials.

Accomplishments: Epidemiological analyses of citrus canker epidemics led to: Development and deployment of the sentinel tree grid survey method for detection of citrus canker outbreaks in urban situations and the development of an International

Phytosanitary Standard for assessing disease presence/absence abroad; and the potential distance of spread of citrus canker which resulted in redefining the citrus canker eradication program. A novel tobamovirus was isolated from symptomatic hibiscus in Florida nursery stock and landscape plantings, characterized and subsequently recognized as a new species, Hibiscus Latent Fort Pierce Virus (HLFPV). Detection and management strategies have been developed, tested and transferred. Research demonstrated the utility of using degenerate primers and PCR to identify genetically distinct components in complex mixtures of Citrus tristeza virus (CTV).

Impact: Distance of canker spread models were the justification for a new eradication law in Florida and along with the sentinel tree survey method that has been used internationally, has become the scientific basis of the citrus canker eradication program. HLFPV and a recently discovered tobamovirus from Singapore are the first well-characterized tobamoviruses known to infect malvaceous hosts, including cotton, okra and kenaf, which necessitate a revision of the tobamovirus genus to accommodate the addition of a new subgroup and extend virological knowledge. Citrus tristeza virus genotype results enhanced regulatory measures concerning identification and management of this pathogen.

Additional information: Research has benefited from continuing collaborations with scientists at other locations and institutions. Major collaborations during this five year cycle have included University of Florida-Citrus Research and Education Center, Florida Department of Agriculture and Consumer Services-Division of Plant Industry and APHIS-PPQ.

Canines were found to be able to differentiate volatile compounds produced by citrus canker-infected trees and may be a viable new detection tool for Citrus canker. The epidemiology of citrus sudden death was examined in Brazil and led to the suspicion that the new disease was probably caused by an insect-transmitted virus similar to tristeza. A protoplast system for viral RNA-dependent RNA polymerase extraction suitable for use with a range of plant viruses was developed. Key West nightshade was identified as a potential long-term maintenance host for TSWV. Requests for seed or cuttings have been received from and materials shipped to locations in Florida, Georgia, Wisconsin, California, Thailand, Canada, Holland and Spain following presentation and publication of this research. Several new hosts of tomato spotted wilt virus have been identified (in Florida, in North America), which will contribute to our understanding of the disease cycle. New genotypes of CTV have been identified, increasing our understanding of the genetic diversity of CTV and providing data for increased control of CTV caused diseases. Research provided identification of aphid transmissible and non-transmissible CTV isolates as a baseline for studying viral genetic control of CTV transmission by aphids. Epidemiological studies of CTV, Huanglungbing, Citrus Sudden Death have provided new control/eradication strategies.

Documentation:

Kamenova, I., Adkins, S. 2004. Comparison of detection methods for a novel tobamovirus isolated from Florida hibiscus. Plant Disease. V. 88 p. 34-40.

Adkins, S., Rosskopf, E.N. 2002. Key West nightshade, a new experimental host for plant viruses. Plant Disease. V. 86 p. 1310-1314.

Hilf, M.E., V.A. Mavrodieve and S.M. Garnsey. 2005. Genetic Marker Analysis of a Global Collection of Isolates of Citrus tristeza virus: Characterization and Distribution of CTV Genotypes and Association with Symptoms. Phytopathology, in press.

R.H. Brlansky, M.E. Hilf, P.J. Sieburth, W.O. Dawson, P.D. Roberts and L.W. Timmer. 2005. 2005 Florida Citrus Pest Management Guide: Tristeza. PP-181, Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Gottwald, T. R., Sun, X., Riley, T. D., Graham, J. H., Ferrandino, F., and Taylor, E. L. 2001. Geo-Referenced, Spatiotemporal Analysis of the Urban Citrus Canker Epidemic in Florida. Phytopathology. 92:361-377.

Hughes, H., Gottwald T. R., and Yamamura, K. 2001. Survey methods for assessment of citrus tristeza virus incidence in urban citrus populations. Plant Disease 86:367-372.

predominant strains.

Grey Leaf Spot of Corn

Background: Gray leaf spot (GLS) of corn, caused by *Cercospora zeae-maydis,* has increased in frequency, distribution, and severity during the past two decades and is the major foliar disease of corn, causing substantial economic losses in the United States, Africa, and South America. The increased incidence and severity of GLS are associated with conservation tillage practices and monoculture of corn, which result in a build up and survival of inoculum in debris for early infection of the subsequent crop. Most commercial hybrids are moderately to highly susceptible to *C. zeae-maydis* and vary widely in their disease reaction from location to location, either as a result of genetic diversity in the pathogen population or major environmental effects. Published estimates of yield reduction due to GLS range from 2% to >40%. Thus, a conservative estimate of 5% yield reduction translates into a loss of over 250 million bushels and an economic impact of several hundred million dollars in the four major corn-producing states in the midwestern U.S. alone (IA, IL, IN, NE), where nearly 50% of the nation's corn is produced. In seed production fields, additional costs are incurred by the application of fungicides used to reduce the damage from *C. zeae-maydis*. Like many species of *Cercospora*, *C. zeae-maydis* produces cercosporin, a low molecular weight, lipid-soluble perylenequinone that localizes in host cell membranes and, thus, is toxic to a diversity of organisms beyond the host range of the producing fungal species. Cercosporin affects plant cells through the light-activated generation of active oxygen species, predominantly singlet oxygen, which results in lipid peroxidation, membrane damage, leakage of nutrients, physical stress, and eventually cell death. The role of cercosporin in GLS had not been determined.

Accomplishment: Previous research by ARS established that GLS of corn is caused by two sibling species of *Cercospora zeae-maydis*. Two genetically distinct but morphologically indistinguishable forms of the pathogen were isolated from diseased leaves of corn in a national GLS survey. Members of one group of the pathogen were found throughout the corn-producing regions of the U.S., whereas members of the other group were localized in the eastern third of the country. ARS analyzed global populations of the GLS pathogen. Results of analyses comparing DNA profiles of the pathogen from Africa and South America indicated that both forms of the pathogen are present in South America, but the pathogen population in Africa is comprised exclusively of the less prevalent form that is localized in the eastern part of the U.S. Because members of the two groups are morphologically indistinct yet cause identical disease symptoms, a rapid molecular (PCR-based) method was developed to distinguish each sibling species as well as other fungal foliar pathogens of corn and sorghum. The method, which detects differences in the nucleotide sequences within the internal transcribed spacer (ITS) regions of ribosomal DNA, reduces the time and steps that are required to identify the pathogen and provides definitive criteria that are applicable without relying on variable and overlapping taxonomic criteria obtained from cultures *in vitro*.

Toward assessing the importance and essentiality of cercosporin production by *C. zeae-maydis*, ARS determined nutritional conditions that induce or suppress cercosporin synthesis in culture and developed methods for genetic transformation and gene disruption of the pathogen. By cDNA subtraction hybridization methods, ARS identified and analyzed genes that are uniquely expressed during cercosporin biosynthesis. Disruption of genes that were predicted to be critical for cercosporin synthesis established that cercosporin is a virulence factor that is essential for the fungus to cause GLS symptoms.

Impact: This information impacts the screening of corn germplasm for GLS resistance and the development of resistant hybrids via traditional breeding or genetic engineering strategies designed to control GLS by conferring resistance to cercosporin. Analysis of the pathogen population will provide clues to the mechanism of change in this fungus and its relatives that are pathogens of other grain crops. Based on information on the global distribution of the pathogen, a tenable hypothesis was proposed to explain the source of the second sibling species in the U.S. and its phylogenetic relationships with other *Cercospora* and *Mycosphaerella* species. The simple and reliable PCR method of distinguishing between the sibling species was shared with other investigators in South America and Africa, who applied the method to identify isolates of the GLS pathogen and determine genetic diversity in resident populations on those continents. The results of this work were featured in several popular press articles. Numerous investigators from the United Kingdom, Africa, Denmark, and South America as well as Mexico and the U.S. requested specific information about the pathogen and its potential impact on disease severity; others requested cultures of the pathogens.

Additional information: Several collaborators assisted with collections of GLS samples of corn from Brazil, Peru, Colombia, Uganda, Zimbabwe, Kenya, and South Africa. A

colleague at Purdue University provided expertise in the population genetic analysis of the GLS pathogen.

Documentation:
Dunkle, L.D., Levy, M. Genetic relatedness of African and United States populations of *Cercospora zeae-maydis*. Phytopathology. 2000. v. 90. p. 486-490.

Shim, W.-B., Dunkle, L.D. Identification of genes expressed during cercosporin synthesis in *Cercospora zeae-maydis*. Physiological and Molecular Plant Pathology. 2002. v. 61. p. 237-248

Septoria Blotch

Background: *Mycosphaerella* is one of the largest genera of plant pathogenic fungi with more than 1,000 named species, many of which are important pathogens causing leaf spotting diseases in cereals, citrus, banana, and eucalypts as well as many soft fruits such as strawberry, and horticultural crops including a range of *Brassica* species. A few species of *Mycosphaerella* cause disease in humans and other vertebrates, so the genus is of interest both for human and plant health. *Mycosphaerella graminicola* causes Septoria tritici blotch (STB) of wheat, one of the most common and economically important plant diseases worldwide. Control of this disease requires a global annual fungicide input of $400 million, mostly in Europe. Fungicide sprays are not economic in most parts of the United States, so growers must rely on host resistance. By the year 2000, four resistance genes had been named. However, none had been mapped in the wheat genome and no molecular markers were available so these genes had not been used extensively by wheat breeders. With no effective means of control, the economic damage caused by *M. graminicola* probably is more than $275 million every year in lost revenue to U.S. wheat growers.

Accomplishment: Populations of wheat plants segregating for resistance genes were tested for susceptibility and also screened with numerous molecular markers. In addition, several other cultivars were analyzed to test whether they could be sources of new resistance genes. All four of the previously identified STB resistance genes were mapped on the wheat genome, and molecular markers linked to each were identified. Testing of other cultivars identified a new resistance gene in a synthetic wheat cultivar. This gene also was mapped and linked molecular markers were identified. An additional gene was identified in an Australian wheat cultivar but has not yet been mapped. The newly mapped resistance genes were then used in gene-expression studies, which revealed that the resistance response begins within a few hours of inoculation and before penetration of the host by the fungus. This is much earlier than was believed previously, which has stimulated numerous follow-up studies in several laboratories worldwide.

Impact: Mapping of these five resistance genes and identifying linked molecular markers represents a major step forward in our ability to combat STB in wheat. Numerous requests for the resistant germplasm (e.g., from the UK, Denmark, Czech Republic, Germany) and for the linked markers were received from wheat breeding

programs worldwide as soon as the information was published or presented at international meetings. Because each gene was on a different wheat chromosome, it should be relatively easy to combine them into a single cultivar with the aid of the linked molecular markers. One of the STB genes mapped near a cluster of genes for resistance to other fungal pathogens, including Fusarium head blight (scab), *Stagonospora nodorum* glume blotch, and stem rust. A collaborative project was started with the wheat breeder at Purdue University to combine each of these resistances to the four pathogens into a single linkage block on wheat chromosome 3BS. The material with mapped resistance genes also is being used to analyze gene expression during the resistance response, as the STB genes originated from all three of the original species that hybridized to generate bread wheat. That work has lead to new insight into the resistance response and identified candidate genes that could be involved in resistance. Those candidates are subjects for additional investigations in several laboratories worldwide.

Documentation:

Ray, S., Anderson, J.M., Urmeev, F.I., Goodwin, S.B. Rapid induction of a protein disulfide isomerase and defense-related genes in wheat in response to the hemibiotrophic fungal pathogen *Mycosphaerella graminicola*. Plant Molecular Biology. 2003. v. 53. p. 741-754.

Background: Plant species that do not become infected by a particular pathogen are known as non hosts. Because most plants are resistant to most potential pathogens, the number of possible non-host resistance genes represents an enormous untapped resource for crop improvement. Utilizing this resource requires a better understanding of non-host resistance and host-pathogen interactions. The Septoria tritici blotch pathogen of wheat, *Mycosphaerella graminic**ola,*** does not infect barley. Instead, barley is host to *Septoria passerinii*, the cause of speckled leaf blotch, which does not infect wheat. However, the evolutionary relationships between these species and among others in the genus *Mycosphaerella* were not known. Progress on understanding non-host resistance depended in part on developing either *S. passerinii* from barley or *M. graminicola* (asexual stage: *S. tritici*) from wheat into a genetic model for species in the genus *Mycosphaerella*, yet very little was known about the evolutionary history or genetics of either species.

Accomplishment: Large-scale phylogenetic analyses were performed of *M. graminicola*, *S. passerinii*, and many other species thought to be related to *Mycosphaerella*, including other grain crop pathogens such as *Cercospora zeae-maydis* and the banana sigatoka pathogen M. *fijiensis*. The results showed that *M. graminicola* and *S. passerinii* were extremely closely related and could serve as good models to analyze non-host resistance interactions in wheat and barley. The analysis also identified a potentially new species of *Septoria* on the wild barley *Hordeum jubatum.* Work as part of a collaborative project with scientists in the Netherlands led to a genetic linkage map for *M. graminicola*, the first for any species in the genus *Mycosphaerella*. The work also lead to cloning of the mating-type genes from *S. passerinii*, which previously had been

thought to be asexual, and to the discovery of active transposable elements (mobile pieces of DNA) within *M. graminicola*.

Impact: The large-scale phylogenetic analyses greatly increased our understanding of evolutionary relationships within the genus *Mycosphaerella*, and indicated that new species probably are stimulated to form by the acquisition of new traits. For example, speciation of *M. graminicola* and its relatives was probably triggered by a host shift from a dicot to a grass host. Speciation of *Cercospora* species probably was initiated by acquisition of the ability to produce the phytotoxin cercosporin. The evolutionary relationships published from this study now have been confirmed in other laboratories worldwide. Work on the genetics of *M. graminicola* has now established this species as a model for its genus and for fungi in the order Dothideales. Based on these accomplishments, a proposal to sequence the genome of *M. graminicola* was picked up last year and the sequencing is being done during early 2005. The impact of the genomic sequence will be enormous and will lead to a global surge of new research on this important plant pathogen. Studies analyzing gene expression during non-host resistance responses of barley to the wheat pathogen were performed based on the results of the phylogenetic analysis and are being prepared for publication.

Additional information: Collaboration with a research group in the Netherlands led to the development of *M. graminicola* as a model genetic organism for the genus *Mycosphaerella,* and to the proposal to sequence the genome of that organism.

Documentation:

Adhikari, T.B., Yang, X., Cavaletto, J.R., Hu, X., Buechley, G., Ohm, H.W., Shaner, G., Goodwin, S. B. Molecular mapping of *Stb1*, a potentially durable gene for resistance to septoria tritici blotch in wheat. Theoretical and Applied Genetics. 2004. v. 109. p. 944-953.

Goodwin, S.B., Waalwijk, C., Kema, G.H.J., Cavaletto, J.R., Zhang, G. Cloning and analysis of the mating-type idiomorphs from the barley pathogen *Septoria passerinii*. Molecular Genetics and Genomics. 2003. v. 269. p. 1-12.

Viral and Nematode Pathogens of Potato

Background: Viruses and cyst nematodes present the potato industry of the Northeast with its most severe regulatory problems and Barley Yellow Dwarf (BYD) continues as the most economically important virus disease of cereal crops worldwide. This research program focuses on the golden nematode (GN), potato virus Y (PVY), potato leafroll virus (PLRV) and the viruses causing BYD. GN is a quarantined pest that can cause direct crop loss, increase pest control costs, constrain cropping patterns, devalue property, and interfere with both domestic and international trade of a wide variety of crops. Both PVY and PLRV cause crop losses, interfere with marketing of the crop, and are major factors in certified seed production. These pathogens have been kept in check by regulatory programs, but the effectiveness of the pathogen control programs has been

jeopardized by recent events. A second race (Ro2) of the GN has become established that is virulent on cultivars resistant to Race 1 (Ro1). Potato cultivars that are symptomless carriers of viruses have hampered the ability of seed certification programs to monitor and restrict the distribution of virus-infected seed. The resulting increase in virus disease has further contributed to the emergence of new, more virulent isolates of the viruses. An immediate objective is aimed at understanding pathogen diversity and providing the regulatory agencies with knowledge and diagnostic tools to identify and monitor the problems.

In addition to regulatory issues, nematode and virus diseases of potatoes and grain crops cause severe crop loss. There is a constant need to modify existing disease management strategies and to develop new disease control measures. Most nematicides previously used to control nematode populations have been withdrawn from usage and alternative control strategies have not been developed. Similarly, effective virus disease control strategies are lacking and limited resistance/tolerance has been identified in germplasm collections. New plant biotechnologies are likely to provide the basis for novel methods of nematode and virus control, but success will be dependent upon a more complete understanding of the fundamental mechanisms of host-nematode interactions and host-virus-vector interactions. Rapid identification and containment of nematode and virus diseases coupled with sustainable disease control options will help the eastern potato and grain industries remain viable and allow them to expand market share.

Accomplishment: Breeding for GN resistance is a long-term process that requires time consuming and labor intensive bioassays to determine if the potato genotypes carry the known resistance genes. A PCR-based assay was developed to detect H_1 gene in potato that confers resistance to Race Ro1 of the GN. The new assay allows the evaluation a hundred or more samples in a single day and can track the H1 gene independent of other resistance genes used in the potato breeding program. In the past 5 years three GN resistant cultivars, Eva, Marcy and Monticello have been released to growers. A new race (Ro2) of the golden nematode that overcomes the resistance to Ro1 was identified in commercial potato fields in 2000. These fields were also infested with the predominant race, Ro1. In cooperation with Cornell University scientists, a potato variety was developed with resistance to both races, however, commercially acceptable cultivars with Ro2 resistance will not be available for another 5-10 years. The current recommended GN management system uses Ro1 resistant cultivars to keep the GN at low densities, but this also selects for an increase in Ro2. An alternative system was developed in which Ro1 resistant cultivars are grown no more than twice in five successive crop years that maintains both races at acceptable risk levels. This alternative management system was instituted in the 2002 crop year on most infested land to maintain the integrity of the golden nematode quarantine. Methyl bromide has been the treatment of choice to disinfest items infested with the golden nematode but legislation to eliminate its use has forced the development of alternative treatments. A steam treatment that is 100% effective in ridding infested equipment of the golden nematode was developed, tested and confirmed in field-scale trials and has replaced methyl bromide.

Barley yellow dwarf is a serious disease problem in winter wheat throughout the southeast and disease control strategies developed in the northern states are ineffective. In collaboration with scientists at Clemson University, new information on disease epidemiology was established and used to develop disease management strategies that incorporate scouting of aphid populations as a principal criteria for pesticide applications. Elimination of prophylactic pesticide applications or applications targeting an aphid population that does not significantly contribute to virus spread provide economic and environmental benefits to the grower. Potato Virus Y (PVY) is a re-emerging disease for the seed potato industry for which there are no effective control measures. Greenhouse and field experiments conducted in collaboration with scientists at Cornell and ARS, Orono, Maine, established a relationship between the time of infection of a potato plant and PVY infection of the tubers. The incidence of tuber infection is also influenced by cultivar and virus strain. This information coupled with information from virus surveys and virus genetic studies is being used to develop a more accurate sampling scheme for testing tubers for virus infection and to improve virus management strategies.

A majority of plant and animal viruses are transmitted by insects, however, the molecular and cellular mechanisms of transmission are poorly understood. Cell biology experiments identified and characterized three cell barriers in aphids that regulate the specificity of luteovirus movement in vector and nonvector aphids. ARS identified that the movement of virus across these barriers is regulated by specific domains of two luteovirus proteins that interact with unidentified receptors in the aphids. Biochemical studies identified virus-binding proteins in aphid species that are efficient vectors of luteoviruese; these proteins were not found in nonvector aphids. These were the first aphid proteins identified that may play a definitive role in the transmission of plant viruses. Further characterization of the proteins may shed some light on how viruses have adapted to use aphids to transport them between plants and furthermore, may suggest novel targets to prevent or reduce an aphids ability to transmit viruses. Clonal populations of an aphid species that differ in their ability to transmit viruses have been used to develop an experimental system to identify the aphid genes involved in virus transmission. Transmission is controlled by several major and minor genes that can be aphid and virus specific. Continuing studies are aimed at the development of diagnostic tools to identify vector populations and to identify genes involved in the transmission process. This work has also identified a nonstructural protein encoded by luteoviruses that regulates virus movement in a host-specific manner. Loss of the protein restricts virus to the initially inoculated tissues. This provides a potential target for developing plants resistant to systemic infection by the virus.

Impact: Potato growers in New York have benefited from new GN resistant cultivars and from newly adopted GN management practices that minimize hardships of potato production in GN quarantine areas. The GN potato cultivars are also grown in other states. Technology transfer of steam cleaning procedure for Golden nematode infested equipment benefits farmers operating within the quarantine areas of New York State. BYDV scouting and management practices have been adopted by wheat growers in the SE United States and have led to decreased pesticide use and increased crop value. A necrotic virus management plan has been developed between the United States and

Canada. The specifics of this plan are based in part on results of virus surveys and subsequent research conducted by this research project. Additionally, the fundamental research findings on vector transmission of viruses, virus movement in plants and mechanisms of resistance to the GN have advanced their respective fields and the information is being used to test new technologies for virus and nematode disease management and control.

Additional information: Outside funding has been received from the Southern Regional IPM Program to suppor the BYDV work. Several grants from the Maine Potao Boared supported the PVY management and surveys of potato viruses affecting the Northeastern potato crop. The New York State IPM program provided support for the research on soilborne viruses affecting winter wheat; this research is in collaboration with the Department of Plant Pathology, Cornell University.

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Fusarium of Cereals

Background: Fusarium head blight is one of the most important diseases of wheat and barley world-wide. Effective and economical control measures for the disease are not currently available. New principles and measures for disease management may be developed by learning more about the spread and pathogenic adaptation of the fungal pathogen and by identifying and characterizing new sources of disease resistance in spring wheat. Spread of the disease pathogen, *Fusarium graminearum*, the appearance of new pathogen populations and their fate in the environment are being measured throughout the United States based on population genetic methodologies. The basis of pathogenic adaptation, those traits and biochemical processes that allow for disease causing ability of the fungus, are being determined using a functional genomics approach. Further knowledge of pathogen ecology and the genetic basis for pathogenicity may be used to develop alternative approaches to disease management and control.

Accomplishment: By understanding the fundamental mechanisms by which the Fusarium head blight pathogen causes disease, a novel, stable, and environmentally sensible disease management practices aimed at interfering with the essential processes of pathogenesis may be developed. Toward this goal, genomic resources for the fungus have been developed. The Cereal Disease Laboratory was one of four international contributors of libraries that served as the basis for ESTs now available in GenBank. A BAC library of the fungus was created and used for the whole genome sequencing of the fungus. A DNA sequence-based genetic map was developed and is available on the web. http://www.broad.mit.edu/annotation/fungi/fusarium/markers.html. Manual annotation of the genome and genome-wide characterization of mutants in the process of pathogenicity is underway.

Impact: The Cereal Disease Laboratory has been at the forefront of efforts to develop publicly available genomic resources for *F. graminearum*, including obtaining funds to sequence the genome and coordinating efforts for manual annotation, functional analysis and the integration of the genetic and physical maps. The recent public release of the whole genome sequence of *F. graminearum* represents a major milestone in molecular biology of plant pathogenic fungi. www.broad.mit.edu/annotation/fungi/fusarium/index.html. This information will be useful to all other scientists who are seeking new ways to control the disease or develop Fusarium-resistant wheat and barley because it provides details of the genes required by the fungus to cause disease.

Additional information: Grants were obtained for developing EST resources and functional analysis of the fungus. A grant also was obtained, in collaboration with the Broad Institute of MIT and Harvard, for whole genome sequencing of one strain of the fungus; the sequence is now available on the web. Another grant was funded for functional genomic analysis and to develop an Affymetrix microarray for *F. graminearum*. Collaborators at Purdue University and Michigan State University were involved in various aspects of these projects.

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Prunus Replant Disease

Background. This project focuses on determining etiology and improved management strategies for key diseases of small fruits and deciduous tree crops. Determine the causal agents of Prunus replant disease (PRD) etiology by correlating the incidence of the disease with quantitative and qualitative shifts in the microbial communities inhabiting the host's roots and surrounding soil and completing Koch's postulates with suspect microorganisms. Both culture-based and culture-independent molecular characterizations of the soil-borne microbial communities were used in field plots where PRD incidence can be manipulatedPeach stunt disease is caused by dual infections of *Prunus necrotic ringspot virus* and *Prune dwarf virus*. Multiple methods for the production of virus-free stock are being examined. In grapevines, young vine decline (YVD) was reported in early 1990s and involved several fungal pathogens. However, we discovered a new virus strain and several new graft-transmissible agents (GTAs) which have significant impacts on vine health and productivity and are associated with YVD. Crown Gall, caused by the bacterium *Agrobacterium tumefaciens* is cited as the number one disease of Walnuts in California. Currently there is no known effective control strategy for Crown Gall on Walnut. The disease is present in all Walnut growing regions of the state and can occur on 80% of the trees in a given orchard causing significant yield, and ultimately, tree loss for the grower. Our initial efforts are two fold: 1) Design and develop robust *Agrobacterium* detection methods and 2) Determine the source and origin of the initial infection of the tree. In the southern range of the Walnut growing region, Deep Bark Canker (DBC) is eliminating the use of a Walnut cultivar that once represented >60% of the Walnut industry. Like Crown Gall, there are no effective control measures for DBC. Consequently, our initial efforts are two fold: 1) Design and develop robust *Brennaria* detection methods, and 2) Determine the source and origin of the initial infection in order to develop control strategies and define the latency period for this pathogen.

Accomplishments: ARS identified the etiology of Prunus Replant Disease (PRD). In numerous trials at multiple sites through out the state, ARS discovered a unique soil-borne microbial community associated with the occurrence of Prunus Replant Disease and is now moving towards the identification of individual members of this PRD inducing microbial community by isolates taken from healthy and diseased roots, and the soil around them. Representative isolates (i.e., hundreds of fungi and bacteria from healthy and diseased plots) have now been identified and are being used, in various combinations, to induce RD in order to complete Koch's postulates. In addition, ARS characterized genetic (AFLP) and pathogenic diversity of *P. cactorum* and *P. citricola*, two pathogens that cause widespread damage on almond, strawberry, and many other California crops. The findings have strong implications for cultivar and rootstock breeding programs.

ARS demonstrated that different peach varieties became infected with peach stunt disease (PSD) at different rates when peach seedlings are planted in close proximity to known peach stunt disease sites. This observed genotype affect on PSD susceptibility will be useful in Peach Tree breeding programs.

Impact: Prunus Replant Disease (PRD). This disease is a significant limiting factor to Almond Production in California, which is more than a billion dollar industry in the state. Our work, for the first time, has clearly shown that the soil-borne microbial community is responsible for this disorder. Perhaps more importantly, ARS has developed several cost effective control strategies for this disorder that are have been accepted by the industry. In addition these new control measures no longer require the use of methyl bromide which was the industry standard as recent as a 3-4 years ago. In addition, several control strategies have been developed which are independent of chemical fumigation.
Peach stunt disease (PSD). The largest impact of virus infections on vegetative growth and yields in peach trees occurs when very young trees became infected. In contrast, delayed infections exhibited by selected peach varieties resulted in reduced disease severity through out the season. Growers should not plant the same, or related, variety next to an existing diseased orchard.

Additional information: Peach stunt disease -Work was done in collaboration with theUC Cooperative Extension, Modesto, CA. Research funds were obtained from the California Cling Peach Commission. Grapevine lethal GTAs - Work was carried out in collaboration with UC Davis that contributed to the discovery of new GTA's in Grape. Research funds were also received from various grant sources (The Improvement Advisory Board, The California Competitive Grant Program for Research in Viticulture and Enology, and CPGR). Rootstock trials have been planted and are currently maintained at the Armstrong Tract, in collaboration with the Department of Plant Pathology, UC Davis.

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Hop Powdery Mildew

Background: In June 1996, despite years of quarantine efforts Hop Powdery Mildew was reported on greenhouse-grown hops in the lower Yakima Valley and in another greenhouse in April 1997 near Toppenish, WA (45 miles distant from the first occurrence). The disease was first observed in the field in June 1997 and by July the disease was found throughout the Washington hop-growing regions. It resulted in the

complete loss of 2000 acres (US $10,000,000) of a highly susceptible variety. Hop Powdery Mildew was not observed in Oregon or Idaho until July 1998. In the Pacific Northwest, annual losses attributed to Hop Powdery Mildew and its management is estimated to be 15% or $411/acre.

Accomplishment: ARS developed a comprehensive disease management program that encompasses an infection risk forecast, cultural practicees, and disease monitoring methods for improved disease management of Hop Powdery Mildew. An infection risk forecaster was made publicly available that provided growers with a 2-week history of the infection risk, the current day's risk and a forecast of the infection risk for each of the coming five days. Weather networks were established through cooperation with commercial entities for access to temperature and rainfall data across the three main U.S. hop-growing regions. The disease forecaster is commercially available in Oregon and Washington. Cultural practices were developed that reduced disease pressure while decreasing economic inputs. Knowledge of the spatial and temporal distribution of the pathogen was used to determine the appropriate timing of cultural practices and other control measures and develop effective monitoring methods.

Impact: In 2001-2004, growers using the model made 2-9 fewer applications than a standard calendar program with less disease while maintaining yield and cone quality. Approximately 75% of susceptible hop acreage is being managed using the infection risk forecaster and cultural practices developed from this research. This research has helped reduce average control costs from $460/A to $196/A resulting in an annual savings of 4.2 million/year for the U.S. hop industry. In June 1996, despite years of quarantine efforts Hop Powdery Mildew was reported on greenhouse-grown hops in the lower Yakima Valley and in another greenhouse in April 1997 near Toppenish, Washington (45 miles distant from the first occurrence). The disease was first observed in the field in June 1997 and by July the disease was found throughout the Washington hop-growing regions. It resulted in the complete loss of 2000 acres (US$10,000,000) of a highly susceptible variety. Hop Powdery Mildew was not observed in Oregon or Idaho until July 1998. In the Pacific Northwest, annual losses attributed to Hop Powdery Mildew and its management is estimated to be 15% or $411/acre.

Additional information: The research on Hop Powdery Mildew was done in collaboration with other researchers at Oregon State University and Washington State University, and private companies (Fieldwise, FoxWeather, Western Farm Service, WillburEllis). Outside funding sources were obtained from Hop Research Council, Washington Hop Commission, Busch Agriculture Resources, Inc., EPA, Dow Chemicals, Inc., Washington Commission on Pesticide Registration and numerous hop growers who donated hop yards for use.

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Sorghum Fungal Pathogens

Background: Fungal pathogens cause major economic losses in sorghum yield and seed quality. Exhaustive understanding of the mechanisms of resistance and host/parasite interactions will enable us to devise sound strategies aimed at minimizing the impact of economically important sorghum diseases; and thereby, ensure continued sorghum productivity in the U.S. In 1997, ergot, caused by *Claviceps africana* was first observed in the United States. Ergot poses a serious threat to both grain sorghum and hybrid seed production fields. Losses in both yield and quality could be enormous in heavy or severe infestation. Presently, no known genetic or biological control exists for ergot. Since ergot was given high priority within the ARS, 1) studies were conducted to develop a reliable inoculation method for mass screening of germplasm, to determine physiological basis for resistance to *C. africana*, to monitor the movement of the pathogen in the sorghum growing regions of Mexico and the United States, and to determine survival of the pathogen under different cultural and environmental conditions. 2) Anthracnose is one of the most important diseases of sorghum and, due to the occurrence of different races of the pathogen, identification of multiple sources of resistance and characterization of these resistant sources is crucial to effectively manage the disease. 3) This project also seeks to identify new sources of resistance to grain mold, head smut, and downy mildew.

Accomplishments: (1) Seven different inoculation methods using nine different sorghum lines planted in different environments were evaluated to identify the most reliable method for ergot resistance screening. A reproducible and effective method for inoculation was identified (involving inoculation of flower heads prior to blooming), and the method was shown to be of great utility in ergot resistance screening. (2) The ability of sorghum ergot pathogen to survive in different environments was conducted in Texas. The study showed that ergot macroconidia can survive in all major sorghum producing areas of Texas. Therefore, it can be concluded that inoculum does not need to move long distances in order to initiate an epiphytotic. However, the study also concluded that incorporating ergot infected debris into the soil after harvest will reduce the number of surviving macroconidia, and thereby reduce the inoculum load for the next planting season. (3) In collaboration with scientists in the USDA-ARS, Area wide Pest Management Research Unit in College Station, Texas the viability of ergot spores in the digestive tracts of adult moths was assessed by plating on water agar the spores recovered from the excreta at 24 h, 48 h, and 72 h after spore ingestion. Both male and female moths can carry viable ergot spores in their guts for up to 72 h after a short feeding period on ergot honeydew and these spores, once excreted by the moths, remain infective. This

work is significant because it indicates that contaminated moths migrating from areas in northern Mexico and southern Texas, where ergot is prevalent, will most likely able to transmit and spread the disease into other sorghum-producing areas of Texas and the central United States.

Impact: This research will identify locations where ergot spores will survive from one growing season to the next. Tillage practices which limit ergot spore survival and head smut incidence will be identified. Effective methods for screening and evaluating sorghum genetic resources will be identified. Work from this project will identify pathotypes of *Colletotrichum sublineolum* specific to a region. Knowledge of the virulence patterns and population dynamics will be useful in the deployment of sorghum with resistance genes against specific races in a region. The ability of mobile insect pests of sorghum to passively and actively transmit ergot from infected to non-infected sorghum plants has been demonstrated. This work will ultimately result in development of effective disease control technology that will enhance U.S. sorghum production efficiency.

Additional information: The USDA-ARS Sorghum Pathology CRIS in the Crop Germplasm Research Unit at College Station, TX, has established cooperative research projects dealing the development of control strategies for economically important sorghum diseases with scientists within Texas A&M University (TAMU), and with *Instituto Nacional De Investigaciones Forestales Agricolas Y Pecuarias*, (INIFAP), Mexico. As a result of these relationships, adequate physical and human resources are available to meet the objectives of this project. Experimental plots are available on the Texas Agricultural Experiment Station (TAES) farms in College Station, Corpus Christi, Beeville, Weslaco, Texas and Rio Bravo, Mexico. Collaborations with scientists within ARS, TAMU, and INIFAP greatly increase the scientific expertise available to meet project objectives.

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Beet Viruses

Background: Economically significant virus diseases which affect the sugarbeet and vegetable **i**ndustries of California, the United States and the world. Soil-borne and insect-transmitted viruses of these crops continue to be significant threats to agriculture. Research goals are directed at ecologically sound management practices for disease control. Techniques and methods applied to achieve these goals involve biological and molecular approaches to elucidate the etiology, epidemiology, pathogenesis and molecular evolution of sugarbeet, vegetable and interrelated crop and weed viruses. Gaining knowledge of host susceptibility, viral pathogenicity, epidemiology and transmission of viral agents is critical to understanding the basic biology of viruses and developing effective control practices. Soil-borne viruses and viruses transmitted by aphids, whiteflies and leafhoppers that infect sugarbeets and vegetables cause some of the most significant yield losses among plant pathogens. New, previously unknown viruses continuously emerge and cause significant economic losses, while older viruses re-emerge to once again impact production. The emergence of new viruses has been exacerbated in recent years by the international movement of plant materials and their vectors, increased farming, farming in new areas, and global climatic changes. The most effective means of virus control continues to be virus resistance in combination with intervention in the introduction and dissemination of viral pathogens. This research is directed at detection and identification of new viruses as they emerge in affected crops, and to address immediate and long-term needs for virus disease management, in efforts to prevent significant crop losses. Specifically, this project focuses on virus disease problems affecting sugarbeet and vegetable industries in California and the United States, with particular emphasis on the economically important soil-borne viruses and insect-transmitted viruses. Research aims to improve plant productivity directly and indirectly through studies that lead to increased knowledge on the etiology, epidemiology, transmission, pathogenesis and molecular evolution of sugarbeet, vegetable and interrelated crop and weed viruses.

Accomplishment: Identification of new rhizomania pathotypes in California: *Beet necrotic yellow vein virus* (BNYVV) is the causal agent of rhizomania disease of sugar beet. The virus is transmitted by the plasmodiophorid *Polymyxa betae*. This fungal vector is able to survive in the soil for at least 15 years by means of its resting spores – cystosori. The disease can only be economically controlled by the use of resistant cultivars. In 2002 ~ 2004 in the Imperial Valley of California resistance to rhizomania disease and BNYVV was compromised. Resistant sugar beet cultivars containing the *Rz1* allele, which reduces virus concentration to very low levels and rhizomania symptom development to a minimum, were severely affected by rhizomania and contained elevated virus levels. Distinct BNYVV isolates from Imperial Valley (IV-BNYVV) were identified from infected sugar beet roots by single local lesion isolation. Because these isolates do not contain RNA-5 as determined by RT-PCR and the banding patterns of single-strand conformation polymorphism analyses, we concluded that the resistance-breaking BNYVV isolates from Imperial Valley had likely evolved from the original existing A-type. The pathogenicity of IV-BNYVV isolates was studied. PCR products from coat protein (RNA-2) and P-25 protein (encoded by BNYVV-RNA-3, involved in symptom expression) of IV-BNYVV isolates were sequenced. Sequence alignments from

both coat protein and P-25 protein revealed only minor amino acid changes compared to the existing A-type of California BNYVV isolates.

Impact: ARS research demonstrated that minor genetic changes may have allowed the A-pathotype to overcome *Rz1*-mediated resistance. This knowledge is critical to development of new strategies for control of rhizomania throughout the world, through both traditional breeding and biotechnology. Furthermore it provides important knowledge that has implications for prolonging the useful life of *Rz1* resistant germplasm in areas where resistance has not yet been overcome by virus evolution. Such rapid (only a few sugarbeet crops after beginning use of resistant germplasm) evolution of resistance-breaking pathotypes indicates it may be necessary to moderate the use of resistant germplasm in order to reduce the rate of emergence of resistance breaking pathotypes in other areas.

Additional information: Lettuce dieback disease, caused by a group of tombusviruses known as either *Lettuce necrotic stunt virus* (LNSV) or *Tomato bushy stunt virus* (TBSV), causes severe stunting, necrosis and death of lettuce plants, resulting in serious economic losses in romaine and leaf lettuce production in nearly all lettuce production areas of California and Arizona. Research has led to the identification of a new tombusvirus species responsible for infection of lettuce and tomato, and detection tools for the identification of this virus and related species. Detection tools were ultimately critical to the development and release of resistant germplasm by our collaborators (ARS, Salinas). In addition, recent studies identified environmental or soil-factors that influence disease development. Studies conducted this past year (FY2004) have identified soil-salinity as a factor contributing to elevated incidence of lettuce dieback symptoms when lettuce plants are grown in LNSV-infested soil. These studies are critical to the management of lettuce dieback disease in infested fields, common throughout all California and Arizona production areas. Outside funding was received from the California Lettuce Research Board

A major focus of ARS is understanding an emerging group of whitefly-transmitted viruses known as criniviruses (Family *Closteroviridae*, Genus *Crinivirus*). Our project has characterized numerous criniviruses over the years affecting tomato, lettuce, melon, sugarbeet and most recently strawberry. These viruses are transmitted by diverse whitefly species including *Bemisia tabaci*, *Trialeurodes vaporariorum* and *T. abutilonea*. Research is focused on biological and molecular characterization of criniviruses, crinivirus epidemiology, disease etiology and effects on yield. Results have led to biological and molecular characterization of *Tomato chlorosis virus*, characterization of vector specificity for *Strawberry pallidosis associated virus* on strawberry, a disease that can lead to severe stunting and yield loss through mixed infection with other viruses and identification of sources of resistance to *Tomato infectious chlorosis virus* in wild germplasm sources. In addition, this research resulted in a CRADA with Agdia, Inc., Elkhart, Indiana to transfer tools for the identification of tomato infecting criniviruses, as well as other viruses. Outside funidn was received from the California Tomato Commission, the California Strawberry Commission, and Agdia, Inc. (CRADA).

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Multiple Pathogens of Cotton

Background: Plant pathogens and nematodes cause annual yield losses of between 11 and 13%. This results in >$800M in losses for U.S. cotton growers per year. In spite of the introduction of new elite varieties, cotton yields have been stagnant for over a decade. The causes of yield stagnation are varied and complex, but the emergence of new pathogens, the spread of recognized pathogens, and the tenacious resiliency of established pathogens are significant factors. Specific examples include the spread of the reniform nematode, the emergence of race 4 of *Fusarium oxysporum* f. sp. *vasinfectum*, recognition of the maladies bronze wilt and South Carolina seed rot, *Rhizopus oryzae* incited seedling disease, and the persistence of seedling disease pathogens. A multi-prong attack strategy is required to combat these problems. This strategy must include the development of resistant varieties, the identification of pathogenic organisms, the acquisition of a knowledge base to understand both the mechanisms of pathogenicity and the factors affecting plant resistance, and the development of effective biocontrol agents.

Accomplishments: ARS discovered that the reniform nematode commonly occurs in severely damaging numbers at a soil depth below that normally sampled in cotton fields (30-135 cm deep). ARS conclusively established that two species of *Pantoea* are etiological agents that cause South Carolina Seed Rot. ARS established a genetic basis for differences in susceptibility to bronze wilt, and introduced a stable molecular marker into a strain of *Agrobacterium* that is implicated in bronze wilt. ARS discovered that

poor seed quality results in seed leachates that stimulate germination of pathogen propagules in the soil that attack the developing seedling, and used this information to develop new technology to reduce the time required to assess cottonseed quality. ARS discovered that *Rhizopus oryzae* is a virulent soil-borne pathogen inciting pre-emergence damping-off in susceptible cotton cultivars. ARS identified a unique and highly virulent strain of *Fusarium oxysporum* f. sp. *vasinfectum* in cottonseed imported from Australia into California as a feed for dairy cows. This strain has devastated cotton production in areas of Australia where it occurs, resulting in 98% yield losses.

Impact: In 2004, ~2% of the U.S. cotton crop was lost to the reniform nematode; acreage infested with this nematode is expected to increase in 2005 and beyond. Our Upland cotton germplasm lines with reniform nematode resistance from *G. longicalyx* and other sources of resistance in primitive cotton accessions provide the seed industry with an opportunity to develop resistant cultivars that would reduce the spread of this nematode problem and decrease yield losses. The depth at which the reniform nematode can survive may explain unpredictable yield responses to fumigation at shallower depths. Sampling protocols used by consultants as a basis for reniform nematode management decisions should be revised. The U.S. National Cotton Collection was surveyed for resistance to the reniform nematode. Based on these findings, two USDA laboratories in Mississippi are currently introgressing resistance from the sources identified at College Station. Mississippi State University and Delta and Pine Land Company are using the nematode tolerance testing protocols that were developed at College Station and Syngenta, Inc. Identification of the causative agent of South Carolina Seed Rot is the first step required to develop strategies to control this disease. When the genetic basis for susceptibility to bronze wilt was reported, several seed companies stopped the sale of these varieties. Development of a molecular marker in the *Agrobacterium* strain implicated in bronze wilt will facilitate studies on its role in this malady. Recognition of poor seed quality as a major factor in seedling disease development, emphasizes the need to use good quality seed. The development of rapid test procedures to determine seed quality will reduce the time for evaluating seed quality by two-thirds. Identification of the highly virulent isolate of *Fusarium oxysporum* f. sp. *vasinfectum* from Australia resulted in the initiation of steps to more thoroughly disinfest cottonseed before it is allowed into the United States.

Cooperating state and private research institutions provided not only the land but all agrochemicals, fuel, equipment, and labor. Syngenta Inc. provided fumigant, insecticides, and labor for planting, harvesting, and sampling.

Additional information: The projects evaluating deeply occurring reniform nematodes involved collaboration with 20 non-USDA entities and two other ARS locations. This research required evaluation of infested farms and comparative field studies in Texas, Louisiana, Arkansas, Mississippi, Alabama, and Georgia. Cooperating entities included eight cotton farmers, Experiment Stations, State Cooperative Extension Services, and industry scientists. Cooperating state and private research institutions provided not only the land but all agrochemicals, fuel, equipment, and labor. Syngenta Inc. provided fumigant, insecticides, and labor for planting, harvesting, and sampling.

Documentation:

Howell, C.R. 2002. Cotton seedling pre-emergence damping-off incited by *Rhizopus oryzae* and *Pythium* spp. and its biological control with Trichoderma spp. Phytopathology. 92:177-180.

Bell, A.A., Wheeler, M.H., Liu, J., Stipanovic, R.D., Puckhaber, L.S., Orta, H. 2003. Studies on polyketide toxins of *Fusarium oxysporum* f. sp. *vasinfectum*: Potential targets for disease control. Pest. Management Science. 59:736-747.

Medrano, E.G., Jones, M., Bell, A.A. 2004. Association of *Pantoea agglomerans* with seed rot of South Carolina cotton. Phytopathology. 94:S69.

Westphal, A., Robinson, A.F., Scott, A.W., Santini, J.B. 2004. Depth distribution of *Rotylenchulus reniformis* under crops of different host status and after fumigation. Nematology. 6(1):97-108.

Discovery Area III: Host/Pathogen/Vector Interactions and Disease Development.

Oxidative Metabolism

Background: Studies of human and animal health are continually revealing that oxidative metabolism plays an important and often controlling role not only in disease resistance but also in various aspects of disease and other stress conditions. As in the animal health field new techniques are needed to study the unique aspects of oxidative metabolism in plant disease. This study focuses on the role of oxidative metabolism, i.e. the production and regulation of prooxidants and antioxidants, in the earliest phases of the plant/pathogen interaction. For bacterial pathogens, the first interaction with the plant generally occurs in the apoplast once the inoculum is absorbed into the tissue; for fungal pathogens the first interaction is often on the plant epidermis where, in many interactions, the apoplast interacts with the infection droplet.

Accomplishment: With the development of new techniques to measure apoplastic antioxidative capacity and plant cell responses, ARS discovered that specific extracellular phenolics are induced in the apoplast and possess bioactive properties that can affect the plant-bacterial interaction. Increased concentration of acetosyringone, an extracellular phenolic, enhances the recognition of the pathogen by the plant cell. The concentration of acetosyringone and other extracellular phenolics is greatly reduced by the production of reactive oxygen species that are often produced in plant bacterial interactions. The concentration of extracellular phenolics is also stimulated by increased concentration of bacteria.

Impact: Previously, the major roles of these phenolics were considered to be 1) antioxidants to counter oxidative stress or 2) structural reinforcement of the cell wall or lignin. This accomplishment demonstrates that apoplastic phenolics can also affect

disease susceptibility or resistance. The balance between prooxidant and antioxidant mechanisms will determine the concentration of the bioactive phenolics and thus influence the plant-bacterial interaction.

Additional information: ARS is currently collaborating with the Russian Research Institute of Phytopathology to study the oxidative metabolism of the infection-droplet/apoplastic microenvironment. The goal is to identify the dominant processes governing the oxidative balance and determine which might be susceptible to manipulation for disease control.

Documentation:

Baker, CJ., Orlandi, E.W., and Deahl, K. 2000. Oxygen metabolism in plant/bacteria interactions: characterization of the oxygen uptake response of plant suspension cells. Physiological and Molecular Plant Pathology. 57:159-167.

Baker, C.J., Mock, N., Deahl, K., Bailey, B., and Roberts, D. P. 2001. Oxidative metabolism in plant/bacteria interactions: characterization of the oxygen uptake response of bacteria. Physiological and Molecular Plant Pathology. 59:17-23.

Baker, C.J., O'Neill, N.R., Deahl, K., and Lydon, J. 2002. Continuous production of extracellular antoxidants in suspension cells attenuates the oxidative burst detected in plant microbe interactions. Plant Physiol. Biochem. 40: 641 – 644.

Baker C.J., Mock N.M. 2004. A method to detect oxidative stress by monitoring changes in the extracellular antioxidant capacity in plant suspension cells. Physiological and Molecular Plant Pathology 64:255-261.

Baker, C.J., N.M. Mock, B.D. Whitaker, D.P. Roberts, C.P. Rice, K.L. Deahl, and A.A. Aver'yanov. Involvement of acetosyringone in plant-pathogen recognition. Biochemical Biophysical Research Communication 328:130-136.

Post-Harvest Disease of Cucurbits

Background: Cucurbits are important horticultural crops in the United States. Pre- and postharvest diseases cause substantial economic losses. Vine decline diseases, caused by various bacteria and fungi, have become yield-limiting in muskmelon, watermelon, and pumpkin in all major production areas over the past fifteen years. Fruit rots can completely destroy the entire harvest as the fruit near maturity. In addition, muskmelons have a limited shelf-life of two to four weeks and exports are limited due to the short shelf-life and postharvest decays. The major emphasis of this project is to provide integrated management for vine declines and determine the mechanisms of latent infections in cucurbit fruit. Infection of the fruit and subsequent decay are related to developmental stage of the fruit. Consequently, we are studying fruit developmental stages and fruit susceptibility to various postharvest pathogens in order to understand why some fungi decay immature fruit and other fungi can only decay fruit that are fully mature.

Accomplishment: Of particular interest to the melon industry is to find a natural inhibitor of fungal-incited postharvest diseases that destroy up to 15% of the annual United States harvested cantaloupe crop. Scientists at South Central Agricultural Research Laboratory, Lane, Oklahoma, set out to demonstrate the existence of natural plant proteins in cantaloupe fruit that fight against fungal infection and to investigate their actions on the enzymes that disease-causing fungi use to destroy the fruit. The production of a natural inhibitor of fungal enzymes was found to be at high levels during the early stages of cantaloupe fruit development decreasing sharply just prior to netting, and then slowly decreasing during fruit ripening with differential expression of active forms of the inhibitor being observed. This information will aid in the development of cantaloupe varieties more resistant to postharvest fungal diseases.

Impact: A novel approach was developed to study latent and non-latent infections to the two processes involved in fruit rot. Information on the potential mechanisms of latent infections in cantaloupe has been published and is available to other researchers. Additional work on enzyme inhibitors is required to be able to fully utilize this technology. However, latent infections in other fruits and vegetables may be controlled by similar mechanisms.

Additional information: Outside funding was received from the National Research Initiative and the Texas/Oklahoma Watermelon Association.

Documentation:

Bruton, B. D., F. Mitchell, J. Fletcher, S. D. Pair, A. Wayadande, U. Melcher, J. Brady, B. Bextine, and T. H. Popham. 2003. *Serratia marcescens*, a phloem-colonizing, squash bug -transmitted bacterium: Causal agent of cucurbit yellow vine disease. Plant Disease. 87:937-944.

Pair, S.D., Bruton, B.D., Mitchell, F., Fletcher, J., Wayadande, A., and Melcher, U. 2004. Overwintering squash bugs harbor and transmit the causal agent of cucurbit yellow vine disease. J. Of Economic Entomology. 97(1):74-78.

Wheeler, M.H., Bruton, B.D., Puckhaber, L.S., Zhang, J., Stipanovic, R.D. 2004. Identification of 1, 8-dihydroxynaphthalene melanin in Monosporascus cannonballus and the analysis of hexaketide and pentaketide compounds produced by wild-type and pigmented isolates of the fungus. Journal of Agricultural and Food Chemistry. 52(13):4113-4120.

Fish, W.W. and Davis, A.R. 2004. The purification, physical/chemical characterization, and cDNA sequence of cantaloupe fruit polygalacturonase-inhibiting protein. Phytopathology. 94:337-344.

Fish, W.W., Madihally, S.V. 2004. Modeling the inhibitor activity and relative binding affinities in crude enzyme - inhibitor-protein systems: insights into developmental regulation. Biotechnology Progress. 20:721-727.

National Sclerotinia Initiative

Background: The National Sclerotinia Initiative, funded by this research project, was implemented in FY2002. The Initiative is a coordinated research effort aimed at reducing the economic threat of Sclerotinia in sunflower, soybean, canola, dry edible beans, chickpeas, lentils, and dry peas. This research consortium of more than 60 federal and state university scientists includes research conducted at 6 ARS labs, 14 land grant universities, and cooperating commodity organizations. Research outside of ARS is managed through the use of Specific Cooperative Agreements. Initiative activities are coordinated by the ARS Center in Fargo, North' Dakota. Research projects are established based on the needs outlined in the Initiative strategic plan and are organized into four research priority areas: a) epidemiology and disease management (including crop production practices & biological/chemical control); b) genomics; c) pathogen biology and development; and d) variety development/germplasm enhancement. Research project selection and progress is currently evaluated on an annual basis by both an outside peer review committee and commodity organization review teams.

Accomplishments: Numerous accomplishments have occurred as a result of the efforts of this research consortium. To date, research and technology transfer activities conducted through the Initiative have resulted in numerous achievements in the areas of genetics and breeding, disease epidemiology, and crop management practices. The most notable accomplishments include:

- Development of a Sclerotinia risk map for dry bean producers and continued validation and expansion of risk maps for canola.
- Identification and characterization of two QTL (genes) that condition partial resistance to white mold in common bean.
- Verification of high levels of white mold resistance in >20 known and 2 new *Phaseo/us coccinues* accessions.
- Investigation of the biochemical basis of white mold resistance in dry bean and identification of oxalate sensitivity as a means to distinguish white mold susceptibility.
- Identification of canola cultivars with improved tolerance to white mold. .
- Establishment of a comprehensive Sclerotinia Initiative web site to serve the needs of the agricultural community and to provide educational information to the general public.
- Identification of six lentil cultivars and one breeding line with relative resistance to white mold.
- Determination of effective fungicides for management of Sclerotinia in sunflower

and
dry edible bean.

- § Identification and evaluation of a biological control agent that shows promise in ' managing white mold in various crops.
- § Identification of wild sunflower accessions suspected to have genetic resistance to Sclerotinia.
- § Development of transgenic expression of a broad-spectrum antifungal peptide in soybean
that confers resistance to Sclerotinia.

Accomplishments: ARS developed a Sclerotinia risk map for dry bean producers and continued validation and expansion of risk maps for canola. ARS established a comprehensive Sclerotinia Initiative website to serve the needs of the agricultural community and to provide educational information to the general public.

Impact: The research conducted under this broad project has had significant impact on improving the current and future management strategies for this important disease. The collective annual losses for the crops participating in the Initiative have been as high as $252 million. Research and technology transfer activities conducted by the Initiative have already begun to have a positive impact by providing new biological and chemical fungicide management tools to reduce annual field losses, by identify new accessions, cultivars, and/or plant varieties with resistance to the disease, and by identifying methods to accurately predict disease incidence and thus provide growers with optimal integrated management tools to address disease infestations.

Documentation:

Auclair, J., Boland, G. J., Cober, E., Graef, G. L., Steadman, J. R., Zilka, J., Rajcan, I. 2004. Development of a new field inoculation technique to assess partial resistance in soybean to *Sclerotinia sclerotiorum.* Can. J. Plant Sci. V. 84 p. 57-64.

Guimaraes, R. L., Stotz, H. U. 2004. Oxalate production by *Sclerotinia sclerotiorum* deregulated guard cells during infection. Plant Physiology. V. 136 p. 3703-3711.

Plum Pox Virus

Background: Exotic plant viruses and their potential insect vectors pose threats to our agricultural system through natural or intentional introductions. Development of detection technologies, identification of causal agents, potential mechanisms of transmission, and determination of factors required for disease management, eradication, or prevention are important to protect our agricultural production systems. Viruses readily mutate and recombine their genetic codes, therefore, understanding dynamics of population change improves the probability of control, prevention, and eradication.

Accomplishment: Since the discovery of plum pox most important virus disease of stone fruits in PAin 1999, there has been an effort to understand the pathway of

introduction, strain(s) of the virus agent, mechanisms of transmission, supplemental host range, and improved detection capabilities. ARS has determined that all isolates belong to one of two clades of strain D of plum pox potyvirus. ARS has developed a very effective, specific real-time PCR platform for rapid identification of any PPV strain. ARS has determined the potential host range of the virus in wild and ornamental Prunus species and determined the role of endemic orchard aphids in local transmission.

Impact: Understanding the molecular nature of plum pox populations provides guidance on the origin of the viral introductions, pathways of movement, and frequency of mutational change. Determination of fruit as an infectious unit has resulted in new recommendations for disposal of cull fruit. Determination of the potential host range of the PA isolates has reduced the quarantine pressure on movement of cherry germplasm and provided insights into what alternative species may be important sources of inoculum influencing the complete eradication of PPV. The real-time PCR primers and probes developed at ARS are being used in the quarantine area in PA for rapid, accurate detection of new isolates of PPV.

Collaborative research with Penn State University (PSU) was significant in the determination of aphids frequenting orchards in PA that were potential vectors of PPV. Aphids were colonized in the insectary at PSU, transported to Fort Detrick, and utilized in transmission experiments in the BSL3-P facility. This collaboration was directed by Congressional mandate in the FY 2001 Appropriation Bill.

Additional information. Considerable progress has been made on two important insect-transmitted pathogenic diseases of citrus. The brown citrus aphid (BCA) is the most efficient vector of severe stem pitting isolates of citrus tristeza virus (CTV). ARS has separated several mild and severe CTV subisolates from mild field isolates of CTV and has re-inoculated sweet orange and Mexican limes with known isolates and re-isolated new, apparently recombinant forms. ARS has indications that the BCA may be selectively transmitting one strain versus others and/or the aphid serves as a bottleneck in separating virus population components. This knowledge will lead to better detection technologies and will allow better screening of citrus germplasm. The glassy-winged sharpshooter is a pest of grape, citrus, and other species in California and Florida. It is a good vector of Pierce's Disease of grape (*Xylella fastidiosa*) and ARS has shown it can vector the *Xylella* that causes citrus variegated chlorosis (CVC), a devastating exotic disease of sweet orange in Brazil and Argentina. Determination of both *Homalodisca coagulata* and *Oncometopia nigricans* (sharpshooters in the United States as potential vectors of CVC makes the establishment of CVC in the United States a more meaningful threat should it be introduced.

Documentation:

Gildow, F., Damsteegt, V., Stone, A., Schneider, W., Luster, D., and Levy, L. 2004. Plum pox in North America: Identification of aphid vectors and a potential role for fruit in virus spread. Phytopathology 94:868-874.

Brlansky, R.H., Damsteegt, V.D., Howd, D.S., and Roy, A. 2003. Molecular analyses of Citrus tristeza virus subisolates separated by aphid transmission. Plant Disease 87:397-401.

Damsteegt, V.D., Brlansky, R.H., Phillips, P.A., and Roy, A. 2005. The glassy-winged sharpshooter, *Homalodisca coagulata* Say, a potential new vector of *Xylella fastidiosa* Wells, causal agent of citrus variegated chlorosis. Plant Disease 89: 0000 (Accepted)

Barley Yellow Virus of Wheat

Background. Increased acreage of reduced tillage practices in cereal growing regions of the United States has resulted in higher incidence of fungal diseases of wheat and barley. Although low-till practices generally do not increase the incidence of virus diseases, these diseases still result in serious yield losses in cereal crops. If given proper environmental conditions these yield losses can reach 20 to 30% or more for both fungal and viral diseases. Because genetic resistance is the most affordable and effective means of disease control, an attempt should be made to identify resistance in segregating populations, wild relatives, synthetic, and other available germplasm for potential introgression into resistant varieties. Also, because host resistance is a result of inhibited pathogen virulence, it is critical that there be an understanding of virulence to better understand the complete host-parasite interaction.

The development and optimal deployment of effective new disease control strategies depends on significant improvement in our understanding of the cellular and molecular interactions that result in resistance, susceptibility, and/or disease development. Improved knowledge of host genetics and mechanisms of resistance is important, as well as is improved knowledge of pathogen genetics and mechanisms of pathogenesis. Finally, a better understanding of host-virus interactions will also aid in developing and optimizing the potential use of viruses as genetic tools and as vectors for the expression of commercially valuable foreign genes in plants.

Accomplishments: The role of the SnTox1 gene (produced by the fungus *Stagonospora nodorum*) in disease has been identified. Previous to our work, SnTox1 had not been reported and therefore neither had its role in disease development. In this study, ARS used the ITMI wheat population to identify QTLs associated with resistance to *S. nodorum* and investigated the role of the toxin in causing disease. This is important because this disease causes a significant amount of damage to the U.S. wheat crop each year and knowledge of the virulence components of the fungus will assist breeders in developing toxin insensitive and therefore more resistant wheat lines.

Genomic regions harboring QTLs for resistance to *Pyrenophora tritici-repentis* race 5 were identified by ARS, and the chromosomal location of the gene conditioning sensitivity to Ptr ToxB (produced by the fungus *Pyrenophora tritici-repentis*) was determined. The toxin-sensitivity gene, designated Tsc2, was mapped to the distal tip of the short arm of chromosome 2B. This gene was responsible for the effects of a major QTL associated with resistance to the race 5 fungus and accounted for 69 percent of the

phenotypic variation. Together, the major QTL on 2BS identified by the toxin insensitivity gene (Tsc2) and a QTL on 4AL explained 73 percent of the total phenotypic variation for resistance to P. tritici-repentis race 5. This work is significant because the results of this research indicate that Ptr ToxB is a major virulence factor, and the markers closely linked to Tsc2 and the 4A QTL can be used for introgression of resistance into adapted germplasm.

Investigation of host selective toxins (HST) produced by fungal pathogens is necessary to better characterize host-pathogen interactions. In collaboration with Steven Meinhardt of the NDSU Dept. of Biochemistry, ARS detected a HST produced by *Stagonospora nodorum* and determined that it plays a significant role in disease. The HST was partially purified and a host gene for sensitivity to the HST was identified. The identification and characterization of pathogen virulence factors and their role in disease is critical to the development of germplasm containing durable resistance.

Mechanisms of virus pathogenicity remain relatively poorly understood. The determinants of barley stripe mosaic virus (BSMV) pathogenicity to barley were mapped and specific codons in the alpha-a gene of BSMV were identified which determine its pathogenicity to barley and oat. ARS had previously mapped the chromosomal location of a BSMV resistance gene, with the rationale that identification of a marker in close proximity to the resistance gene would be a first step toward the cloning and characterization of the resistance gene. Together, results will provide the foundation for interpreting gene-for-gene interactions between viruses and their hosts, will help elucidate the interactions among viral genes, and will provide a foundation for discovering the mechanisms of viral pathogenicity and host resistance. This in turn will provide for the future design of effective, molecular approaches to disease control.

Impact: Markers identified can be used for introgression of resistance into adapted germplasm, leading to improved yields and quality. Host resistance to fungal diseases may be to the fungi themselves, fungal-produced toxins, or both. Knowledge of the virulence components of pathogenic fungi will assist breeders in developing toxin insensitive and therefore more resistant wheat lines. The identification and characterization of pathogen virulence factors and their role in disease is critical to the development of germplasm containing durable resistance. Generally, this research has resulted in an improvement of our understanding of host-pathogen interactions and the underlying mechanisms involved in the development of disease. Such information is essential in providing the foundation of knowledge needed to facilitate the development of future novel control strategies.

Documentation:

Liu, Z., Faris, J., Meinhardt, S., Ali, S., Rasmussen, J., Friesen, T. 2004. Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin by *Stagonospora nodorum*. *Phytopathology* v.94 p.1056-1060.

Liu, Z.H., Friesen, T.L., Meinhardt, S.W., Ali, S., Rasmussen, J.D., Faris, J.D. 2004. Quantitative trait loci analysis and mapping of resistance to *Stagonospora nodorum* leaf blotch in wheat. *Phytopathology* v.94 p.1061-1067.

Friesen, T.L., Ali, S., Kianian, S., Francl, L.J., Rasmussen, J.B. 2003. Role of host sensitivity to Ptr ToxA in development of tan spot of wheat. *Phytopathology* v.93. p. 397-401.

Barley Yellow Dwarf

Background: Barley yellow dwarf is a serious disease problem in winter wheat throughout the southeast and disease control strategies developed in the northern states are ineffective. In collaboration with scientists at Clemson University, new information on disease epidemiology was established and used to develop disease management strategies that incorporate scouting of aphid populations as a principal criteria for pesticide applications. Elimination of prophylactic pesticide applications or applications targeting an aphid population that does not significantly contribute to virus spread provide economic and environmental benefits to the grower. Potato Virus Y (PVY) is a re-emerging disease for the seed potato industry for which there are no effective control measures. Greenhouse and field experiments conducted in collaboration with scientists at Cornell and ARS, Orono, Maine established a relationship between the time of infection of a potato plant and PVY infection of the tubers. The incidence of tuber infection is also influenced by cultivar and virus strain. This information coupled with information from virus surveys and virus genetic studies is being used to develop a more accurate sampling scheme for testing tubers for virus infection and to improve virus management strategies.

Accomplishments: A majority of plant and animal viruses are transmitted by insects, however, the molecular and cellular mechanisms of transmission are poorly understood. Cell biology experiments identified and characterized three cell barriers in aphids that regulate the specificity of luteovirus movement in vector and nonvector aphids. We identified that the movement of virus across these barriers is regulated by specific domains of two luteovirus proteins that interact with unidentified receptors in the aphids. Biochemical studies identified virus-binding proteins in aphid species that are efficient vectors of luteoviruese; these proteins were not found in nonvector aphids. These were the first aphid proteins identified that may play a definitive role in the transmission of plant viruses. Further characterization of the proteins may shed some light on how viruses have adapted to use aphids to transport them between plants and furthermore, may suggest novel targets to prevent or reduce an aphids ability to transmit viruses. Clonal populations of an aphid species that differ in their ability to transmit viruses have been used to develop an experimental system to identify the aphid genes involved in virus transmission. Transmission is controlled by several major and minor genes that can be aphid and virus specific. Continuing studies are aimed at the development of diagnostic tools to identify vector populations and to identify genes involved in the transmission process. This work has also identified a nonstructural protein encoded by luteoviruses that regulates virus movement in a host-specific manner. Loss of the protein restricts virus to the initially inoculated tissues. This provides a potential target for developing plants resistant to systemic infection by the virus.

Impact: BYDV scouting and management practices have been adopted by wheat growers in the SE United States and have led to decreased pesticide use and increased crop value. A necrotic virus management plan has been developed between the United States and Canada. Additionally, the fundamental research findings on vector transmission of viruses, virus movement in plants have advanced their respective fields and the information is being used to test new technologies for virus and nematode disease management and control.

Additional information: Outside funding was received from the Pennsylvania State University and Cornell University for research on luteovirus transmission by aphids and from Boyce Thompson Institute and the University of Arizona to support research aimed at identifying the genes in aphids that regulate virus transmission.

Documentation:

Lee, L., Kaplan, I. B., Ripoll, D. R., Liang, D., Palukaitis, P., Gray, S. M. 2005. A surface loop of the potato leafroll virus coat protein is involved in virion assembly and aphid transmission. J. Virology 79:1207-1214

Lee, L., Palukaitis, P., Gray, S.M. 2002. Host-dependent requirement for the Potato leafroll virus 17-kDa protein in virus movement. MPMI 15:1086-1094

Gray, S. M., Smith, D. M., Barbieri, L. and Burd. J. D. 2002. Virus transmission phenotype is correlated with host adaptation among genetically diverse populations of the aphid, Schizaphis graminum. Phytopathology 92: 970-975

Magnaporthe of Rice

Background: ARS is studying the molecular biology of host recognition of the model plant pathogenic fungus, *Magnaporthe grisea,* by rice. This fungus is considered a premier example of an ascomycete fungal plant pathogen, which can be manipulated both genetically and with the tools of molecular biology. Likewise, rice is considered to be the model monocot plant with a small, diploid genome that can be readily modified by transformation. The genomes of both organisms have been sequenced. Rice Blast Disease caused by *M. grisea* is the most important disease of rice worldwide causing 11-30% loss in yield and reducing grain quality in many rice-growing regions of the world. In the United States rice blast is a sporadic but important problem when it occurs. For example, leaf blast disease has been found in an estimated 400,000 of 1.5 million acres of rice planted in Arkansas this year. Rice is the staple crop of two thirds of the human population and major increases in population are expected in rice-consuming countries over the next century. Thus rice blast disease is a significant constraint to global food security and agricultural trade.*M. grisea* causes also attacks other important cereals and grasses such as wheat causing wheat blast and perennial rye causing grey leaf spot. Similar genes are often required for infection of different host plants by Magnaporthe. Thus we can anticipate information that is broadly applicable to fungal pathogenesis of grasses will be obtained through studies of this model fungus and its interaction with rice. The evolutionary relationship of fungal host and cultivar specificity genes and their

corresponding disease resistance loci is currently unknown and may be exploited to develop new disease resistance specificities using transgenic and wide hybridization technologies. The use of the cloned *AVR1-CO39* gene as an adjuvant to promote plant health remains largely unexplored.

Accomplishment: The most effective method for control of rice blast is to grow disease resistant plants. Unfortunately, *M. grisea* is able to overcome this resistance within 1-3 years after resistant plants are cultivated widely. ARS is trying to understand the molecular details of how the rice blast fungus is recognized by rice plants that are resistant to blast and how the fungus changes in order to overcome this recognition. In order to improve our understanding of this host-parasite interaction, ARS has cloned a pathogen gene AVR1-CO39 that is involved in recognition of the pathogen by the resistant rice plant. This is the second AVR (AViRulence) gene to be cloned from the rice blast fungus. ARS employed a combination of physical, genetic and molecular genetic methods to obtain the cloned gene. These methods included a high density genetic map and molecular karyotype, targeted genome cleavage methods, and a transformation system that ARS developed for M. grisea that is based on drug resistance. Considerable insight was gained about the map-based cloning approaches through this effort. These approaches and insights will likely have broad application to other organisms such as the cereal rusts in which map-based cloning approaches are being used to clone similar genes. In parallel work, ARS mapped and cloned the corresponding disease resistance locus Pi-CO39 (t), which allows the host to recognize strains of the fungus that carry AVR1-CO39. This work has shown that resistance is controlled by a dominant locus mapping to chromosome 11 of rice. The complete DNA sequence of the locus from two rice genotypes has been completed, annotated, and analyzed for transcriptional activity. Annotation of 416 kb of DNA sequence from rice variety Nipponbare and 231 kb from variety CO39 linked to Pi-CO39(t) locus, that contains the putative receptor for the M. grisea avirulence gene AVR1-CO39, revealed the presence of several NBS-LRR, receptor kinase, and Serpin genes in both haplotypes. The long term goal of ARS is to study the molecular biology of the interaction of Avr1-CO39p and the host resistance gene product and/or other host products in order to understand when, where and how the fungus communicates its presence to the host. Tissue culture methods for induction of callus from rice leaf explants and production of plantlets from this callus have been developed as a first step to transform the plastid. Using this technology, transfer and containment of host resistance and pathogen avirulence genes to rice can be achieved.

Impact: Work accomplished in years one and two of this project (2002-2004) has led to the identification of the polypeptide component of AVR1-CO39 that elicits a PiCO39 (t)-dependent response and the tentative identification of two genes at the PiCO39 (t) locus that function in the disease resistance response in rice. Based on this information, ARS will develop new strategies for engineering novel kinds of broad-spectrum resistance to M. grisea in rice and other cereals and grasses of economic and ornamental value. A novel tissue culture method for the isolation of callus from leaf explants of rice has been developed as a first step toward transformation of rice plastids. The AVR gene has been licensed to Kansas State University for collaborative studies to deliver the gene product

to rice from rice-associated bacteria.

Additional information: Extramural funding for a visiting postdoctoral scientist who was employed by the University of Wisconsin and a graduate student of the University of Wisconsin was key to the discoveries made since the current CRIS project funding does not provide sufficient funding for hiring beyond student hourly employees and a technician. In addition, the University of Wisconsin largely paid for the massive amount of DNA sequence data that was acquired for rice. Microarray technology is being used to study transcript profiling during infection by Magnaporthe and identify DNA polymorphisms for genetic analysis in finger millet, and has been supported by extramural funding and a SCA. Extramural funding to idenify DNA polymorphisms for genetic analysis of QTLs controlling milling quality and sheath blight resistance in U. S. rice varieties is newly supported by the NRI program for applied genomics.

Documentation:
Farman, M. L., Eto, Y., Nakao, Y., Tosa, Y., Nakayashiki, H., Mayama, S., and S. A. Leong. Analysis of the structure of the *AVR1-CO39* avirulence locus in virulent rice-infecting isolates of *Magnaporthe grisea*. Mol. Plant-Microbe Interactions. 2002. V. 15. p. 6-16.

Chauhan, R. S., Farman, M. L., Zhang, H.-B., and Leong, S. A. Genetic and Physical Mapping of a Rice Blast Resistance Locus, *Pi-CO39(t)*, Corresponding to *AVR1-CO39* of *Magnaporthe grisea* Mol. Gen. Genomics. 2002. V. 267. p. 613-617.

Nematode and Viral Pathogens of Soybean

Background: Soybean is an important crop for producers and for consumers. Soybean is used directly for human consumption, as a protein supplement in animal feed, and in an increasing number of industrial applications. Each year, the quantity and quality of soybean produced in the United States is adversely affected by a wide range of pathogens and pests, which cause crop loss of approximately $ billion annually. Disease causing pathogens are being characterized in order to produce new soybean lines that are resistant to diseases. Investigations are being done to identify host genes that permit the vertical transmission of viruses and virus genes that reduce seed quality. Sustainable methods for disease management that incorporate biological controls and conservation tillage are being developed. These investigations will reduce the impact of diseases on soybean production by enhancing soybean germplasm with resistance to pathogens, by creating knowledge of the host-pathogen interactions that lead to disease, and by developing sustainable agricultural practices.

Accomplishment: Soybean cyst nematode is able to overcome all known sources of resistance. A genetic linkage map was developed for soybean cyst nematode and markers for virulence to some sources of resistance were located on the map.

Impact: The genetic linkage map will provide for map-based cloning of virulence genes of soybean cyst nematode and will provide the framework for whole genome sequencing.

This genetic linkage map is the second one developed for a plant-parasitic nematode and the first developed for SCN. This ground breaking work is the most important research done thus far to understand the genetics of SCN, especially parasitic ability and defeating resistance

Accomplishment: Seed-borne virus infections can be an important source of virus inoculum for the establishment of virus diseases in soybean. To identify soybean and virus genes that are important for transmission of Soybean mosaic virus (SMV) through seed, we evaluated the seed transmissibility of eight SMV isolates in seven soybean accessions. A strong correlation was found with aphid transmissibility and specific amino acid sequences in two SMV proteins

Impact: These findings suggest that the same genetic elements are involved in vertical and horizontal transmission of the SMV isolates analyzed in these studies. Resistance to viruses is often strain specific, which necessitates the deployment of resistance genes appropriate for highly adaptive virus populations. If seed-transmitted soybean-infecting viruses invade embryos by similar pathways, the identification of virus and host genes involved in transmission of viruses through seed will lead to the development of more generic strategies to limit the impact of seed-borne infections as sources of virus epidemics.

Additional information: Collaborative efforts with colleagues from the University of Illinois and outside funding provided personnel and resources to produce the first genetic linkage map of soybean cyst nematode and to place markers for virulence on the linkage map.

Documentation:

Atibalentja, N., Bekal, S., Domier, L. L., Niblack, T. L., Noel, and Lambert, K. N. A genetic linkage map of the soybean cyst nematode, *Heterodera glycines*. Molecular Genetics and Genomics. (Accepted 12/29/2004).

Domier, L. L., Latorre, I. J., Steinlage, T. A., McCoppin, N. K., Hartman, G. L. 2003. Variability and transmission by *Aphis glycines* of North American and Asian *Soybean mosaic virus* isolates. Archives of Virology v. 148 p. 925-1941.

Harrison, B., Steinlage, T. A., Domier, L. L., D'Arcy, C. J. 2005. Incidence of *Soybean dwarf virus* and identification of potential vectors in Illinois. Plant Disease v. 89 p. 28-32.

Potato Spindle Tuber Viroid

Background: Viroids are parasites of higher plants that are far smaller and simpler than conventional plant viruses - small, highly structured, circular RNA molecules which lack a protective protein capsid or mRNA activity. Viroid diseases are responsible for significant losses of food and fiber, for example in potato, citrus, and tomato, but intensive efforts have failed to identify useful sources of conventional genetic resistance. This research is gathering the fundamental knowledge needed to produce plants that are

resistant to viroid disease while also addressing practical problems and opportunities created by viroid infection. Specifically, ARS is attempting to i) identify molecular interactions mediating viroid movement in the host vascular system, ii) determine the role of a viroid-induced protein kinase in the disease process, iii) identify structural features responsible for viroid entry into the nucleus or chloroplast, and iv) develop and test new strategies to increase citrus production efficiency.

Accomplishments: Potato spindle tuber viroid (PSTVd) replicates in the nuclei of infected host cells. Using *Potato virus X* as a gene expression vector, ARS has shown that adding sequences derived from PSTVd to a non-translatable mRNA encoding green fluorescent protein (GFP) causes the mRNA to be taken up into the nucleus where the intron is removed and the now-functional mRNA is released to the cytoplasm. The appearance of green fluorescence in leaf tissues inoculated with GFP constructs containing a full-length PSTVd molecule embedded in the intron verifies that nuclear import and RNA splicing did occur.

Several viroids including *Potato spindle tuber viroid* (PSTVd) are currently subject to quarantine regulation. Using a variety of molecular techniques, ARS was able to show that seed potatoes from a Maine seed potato producer suspected to be viroid-infected by Canadian officials were, in fact, healthy. Later collaborative studies with colleagues in the Dutch Plant Protection Service demonstrated that tomatoes growing in commercial greenhouses may be infected by any of four different viroids; i.e., PSTVd, CEVd, TCDVd, and CLVd. Yield reduction data from field experiments clearly showed that CEVd, CLVd and PSTVd have similar effects on potato.

Impact: Current strategies to control the expression of foreign genes in transgenic plants often use tissue-specific promoters to control mRNA synthesis. PSTVd and other viroids provide a rich source of molecular signals that plant scientists can add to foreign mRNAs to regulate their translation and redirect the corresponding proteins to specific subcellular compartments. This opens numerous possibilities for plant biotechnology, for example the possibility of increasing the expression of foreign proteins by moving their mRNAs from the cytoplasm to the chloroplast without the need to transform the chloroplast genome.

Several viroids including PSTVd are currently subject to quarantine regulation. APHIS and state of Maine officials used our data to persuade Canadian authorities to rescind a ban on importation of Maine seed potatoes, thereby preserving a $20 million/year industry. Biological characterization of four different viroids isolated from tomatoes growing in commercial greenhouses, especially their potential effects on both potato and tomato, has pointed out the need to reconsider their phytosanitary risk and quarantine status.

Additional information: Outside funding from the ARS Potato Research Program [FY2004-2005] is being used to support a collaborative study with scientists at the International Potato Center (Lima, Peru) to investigate “Effects of Potato Mop Top Virus Infection on Major U.S. Potato Varieties”. When the first U.S. outbreak of PMTV was

reported in 2002, the potential effect on U.S. potato varieties was largely unknown. The virus is endemic in portions of the Andean region, so Peru is a logical place to carry out such studies. Final results will be available later this year, and the methods developed could be used in the future to screen the CIP germplasm collection for possible sources of resistance to PMTV infection.

Documentation:

Zhao, Y., Owens, R. A., and Hammond, R. W. 2001. Use of a vector based on *Potato virus X* in a whole plant assay to demonstrate nuclear targeting of *Potato spindle tuber viroid*. J. Gen. Virol. 82:1491-1497.

Zhu, Y., Qi, Y., Xun, Y., Owens, R., and Ding, B.2002. Movement of potato spindle tuber viroid reveals regulatory points of phloem-mediated RNA traffic. Plant Physiol. 130:138-146.

Verhoeven, J.Th.J., Jansen, C.C., Willemen, T.M., Kox, L.F., Owens, R.A., and Roenhorst, J.W. 2004. Natural infection of tomato by *Citrus exocortis viroid*, *Columnea latent viroid*, *Potato spindle tuber viroid*, and *Tomato chlorotic dwarf viroid*. Eur. J. Plant Path. 110:823-831.

Suden Oak Death

Background: Sudden oak death (SOD) is caused by *Phytophthora ramorum*, a newly described species found since 1993 to attack ornamentals in Germany and the Netherlands. The same pathogen (as determined by morphology and isozyme analysis) was observed since the mid-1990's to be responsible for death of thousands of oaks in California. Sudden oak death has become of major concern to the ornamentals industry and other agricultural industries because of state, Federal, and international quarantines (Canada) affecting the movement of susceptible, high-value nursery crops such as rhododendrons and azaleas as well as firewood, and other plant-related products. The list of hosts of *P. ramorum* is now over 30 in number and growing, but little is known about species growing in the Eastern United States or their susceptibility. In addition, little is known about the biology of *P. ramorum*, its capacity for survival and spread, or its ability to colonize plant root systems. Major efforts are underway to prevent *P. ramorum* from becoming established in the Eastern United States after several west coast nurseries unknowingly shipped thousands of infected plants throughout the United States in 2003 and 2004.

Accomplishment: ARS determined levels of susceptibility of important plant species to *P. ramorum*, developed a diagnostic assay for the pathogen, and elucidated key unknown areas in *P. ramorum* biology. Over 50 ericaceous ornamentals prevalent in the Eastern United States were evaluated for susceptibility to *P. ramorum* and documented symptoms produced on each host. ARS found that *P. ramorum* is able under specified conditions to infect 11 different Eastern forest species. A PCR-diagnostic assay was developed based on mitochondrial gene sequences for detecting *P. ramorum* with a high level of sensitivity and specificity. *P. ramorum* was also able to infect foliage of 8 oak species

tested, even though foliar disease is not commonly observed in nature for species such as *Q. agrifolia* (coast live oak). ARS also determined disease reactions of *P. ramorum* on other important horticultural species such as camellias and lilacs. In addition, ARS discovered that *P. ramorum*, hitherto considered a disease of above-ground plant parts, can colonize roots of certain ornamental plant species. As part of the studies of root colonization by *P. ramorum*, ARS determined that the thick-walled resting spore (chlamydospore) of *P. ramorum* can persist for over 200 days in nursery potting mix.

Impact: Images of *P. ramorum* symptomatology on many Eastern United States plant species which were produced were disseminated to many state and federal workers and were used in training workshops, National Pest Alerts, and placed on numerous websites. The finding that *P. ramorum* can infect plant roots was a surprising result which radically alters our view of this disease and has resulted in reevaluation of quarantine, exclusion, and eradication protocols directed at preventing the spread of the disease to the Eastern United States. These discoveries have provided information which is assisting Federal and State workers in designing detection protocols and quarantine procedures for nursery stock in light of the recent eastward migration of *P. ramorum*.

Documentation:

Bonde, M. R., Nester, S. E., Olson, M. W., and Berner, D. K. 2004. Survival of teliospores of *Tillletia indica* in Arizona field soils. Plant Disease 88:804-810.

Tooley, P. W., Kyde, K. L., and Englander, L. 2004. Susceptibility of selected ericaceous ornamental host species to *Phytophthora ramorum*. Plant Disease 88:993-999.

Brown Spot of Bean

Background: The quality of crops by identifying genes involved in agronomically important traits such as disease resistance and incorporating these genes into breeding programs. Virus-induced gene silencing (VIGS) is a tool with tremendous potential for identifying these genes.

Accomplishments: The model organism of study is *P. syringae* pv. syringae, the causal agent of brown spot disease of bean. ARS established that the *gacS* and *gacA* genes are required for disease on bean and we have characterized numerous genes within the *gacS/gacA* regulon. ARS completed sequencing of the 28 kb tabtoxin biosynthetic region, this toxin is highly damaging to plants and is regulated by *gacS/gacA*. ARS developed methodologies to uniquely mark bacteria with antibiotic resistances for study in the field. ARS successfully applied VIGS as a tool for genetic analysis in tomato and we have developed methodologies for the quantitation of gene expression in plants using Real-Time qrt-PCR.

Impact: Analysis of the *gacS/gacA* regulon impacts research focused on understanding the genetics of disease caused by both plant and animal pathogens. Development of a

unique method to "mark" bacterial strains with easily distinguishable antibiotic resistances allows the study of the epidemiology of bacterial diseases in the filed environment and allows for the improvement in sampling techniques to detect disease. The sequence of the tabtoxin biosynthetic cluster is of major interest to scientists studying antibiotic resistance mechanisms. The development of Real-Time qrt-PCR techniques to analyze VIGS in plants allows ARS to use this powerful method to study gene action in tomato and potato. The ultimate goal is to improve disease resistance in agronomically significant crops through the identification of important genes. These identified genes can then be used in breeding programs to produce disease resistant cultivars.

Documentation:

Hirano, S. S., D. K. Willis, M. K. Clayton, and C. D. Upper. Use of an intergenic region in *Pseudomonas syringae* pv. syringae B728a for site-directed genomic marking of bacterial strains for field experiments. Applied and Environmental Microbiology 2001. v. 67. p. 3735-3738.

Kinscherf, T. G., S. S. Hirano, and D. K. Willis. Transposon insertion in the *ftsK/spoIIIE* gene impairs *in planta* growth and lesion forming abilities in *Pseudomonas syringae* pv. syringae B728a. Molecular Plant-Microbe Interactions 2000. v. 13. p. 1263-1265.

Kinscherf, T. G., and D. K. Willis. Global Regulation by *gidA* in *Pseudomonas syringae*. Journal of Bacteriology 2002. v. 184. p. 2281-2286.

Willis, D. K., J. J. Holmstadt, and T. G. Kinscherf. Genetic evidence that loss of virulence associated with g*acS/gacA* mutations in *Pseudomonas syringae* B728a does not result from effects on alginate production. Applied and Environmental Microbiology 2001. v. 67. p. 1400-1403.

Willis, D. K., and T. G. Kinscherf. Global Regulation in *Pseudomonas syringae*. Ramos, J. L., editor Kluwer, New York, NY. The Pseudomonads Vol. II. Virulence, gene regulation and metabolism. 2003. p. 223-238.

Citrus Tristeza Virus

Background*:* *Citrus tristeza virus* (CTV) is a phloem-limited closterovirus transmitted by aphids in a semipersistent manner. CTV infects only citrus and citrus relatives and replicates in infected trees as a population of genetic variants. As such, the virulence of CTV stains can vary widely. Endemic strains of CTV in the U.S. mainland are relatively mild and contain only a portion of the biological biodiversity of CTV worldwide. Throughout Asia and Southeast Asia, Australia and New Zealand, the Pacific Islands, South Africa, and South America, highly virulent CTV strains are common and are associated with the efficient CTV vector, *Toxoptera citricida*. This aphid was accidentally introduced into Florida in 1995 and its establishment presents a threat to the U.S. citrus industry. It is now critical to determine how aphid vector populations interact

with CTV populations to spread the disease agent and to affect genetic diversity and symptom phenology of CTV. With new knowledge gained in this area, new control strategies for CTV can be developed.

Accomplishment: Representative isolates of CTV from various citrus fields in Central California were collected and were determined to be mild (e.g., did not induce stem pitting in sweet orange or grapefruit) and did not induce symptoms in commercial citrus on CTV-tolerant or resistant rootstocks. The genomes of these isolates were all found to be the T30 genotype similar to the T30 mild isolate from Florida. These isolates exhibited a range of aphid transmissibility by the cotton aphid, *Aphis gossypii*, from low (0 to 5%) to high (30 to 62%). *Toxoptera citricida* transmitted these isolates with higher efficiency, thus, proving that it was a better vector than *A. gossypii* for California CTV isolates. Sequence comparisons of selected regions of the CTV genome did not show marked differences between the parent isolate and the aphid-transmitted isolates. Furthermore, no differences were noted in deduced amino acid sequences between an isolate when transmitted by *A. gossypii* or *T. citricida*.

Impact: Since the current diversity of CTV in central California appears to be relatively low, the CTV population would probably remain stable for the first few years if *T. citricida* were introduced into California. Changes in the diversity of CTV strains would likely change as a result of recombination and/or mutations of existing genomes rather than the releasing of latent virulent strain(s).

Additional information: A USDA, CSREES Citrus Tristeza Research grant was received with UC Davis on a project comparing transmission of CTV by *Toxoptera citricida*, *Aphis gossypii*, and *Aphis spiraecola*. This research has fostered joint molecular evaluations of CTV isolates transmitted by the different vectors and will help assess the tristeza problem in California if the brown citrus aphid becomes established.

Documentation:

Satar, S., and Yokomi, R. K. 2002. Effect of temperature and host on development of *Brachycaudus schwartzi* (Homoptera: Aphididae). Ann. Entomol Soc. Am. 95:597-602.

Yokomi, R. K., Polek, M., Satar, S., and Gottwald, T. R. 2002. An economic sampling protocol for locating *Citrus tristeza virus* reservoirs in a large area. P. 54-59. In: Proc. 15th Conf. IOCV, IOCV, Riverside.

Gottwald, T. R., Abreu-Rodriguez, E., Yokomi, R. K., Stansly, P. A., and Riley, T. K. 2002. Effects of chemical control of aphid vectors and of cross protection on increase and spread of *Citrus tristeza virus*. P. 117-130. In: Proc. 15th Conf. IOCV, IOCV, Riverside.

Yokomi, R.K. 2004. Transmissibility and genotype analysis of central California isolates of *Citrus tristeza closterovirus.* Phytopathology 94: S131.

Host Plant Resistance to Disease Component

ARS research accomplishments summarized here are separated into three areas related to advancing our knowledge and enhancing our ability to discover, develop, and deploy disease resistance. These discovery areas are: (1) development and/or release of improved of germplasm or varieties with resistance to threatening diseases that reduce yield or quality; (2) development of molecular markers to facilitate selection of resistant germplasm and enhance our understanding of the plant genome; and (3) fundamental studies that address molecular or biochemical mechanisms of resistance to pathogens.

Discovery Area 1: Germplasm Development and Variety Improvement

Background: Host plant resistance to diseases is often lacking or not well developed in many plant species. Increased research to identify and characterize genes for resistance in crop plants, in closely related plant species, or in alien species is needed to enhance opportunities for developing host plant resistance. Research on incorporating such genes into commercially acceptable varieties will lead to new methods of using this cost-effective, environmentally safe disease management strategy.

Cereal Viruses

Background: Small grain cereals are the world's most important food crops and the demand worldwide, is estimated to increase 50% by 2020. Cereals are vulnerable to a biologically diverse group of pathogens and losses to disease are a very significant obstacle to increasing cereal production. Incorporating highly effective and stable host-encoded resistance to pathogenic fungi and important viral pathogens, specifically Barley and Cereal Yellow Dwarf Viruses, is a major component in meeting this demand. Consequently, this research is focused on the identification, characterization and use of genetic loci present in the gene pool of cultivated small grains and related plants that control fungal and viral pathogens. To utilize disease resistance loci effectively through conventional breeding requires identifying DNA markers linked to these loci and understanding the genetic, molecular and biochemical basis of this resistance in cereal crops.

Accomplishment 1: Cultivated wheat does not have effective resistance to Barley and Cereal Yellow Dwarf Viruses (YDV). Resistance, derived from a related wheatgrass *Thinopyrum intermedium*, was integrated into elite wheat lines and two YDV resistant wheat cultivars were released. The parental line P961341 was also registered as a YDV resistant germplasm line. This YDV resistance provides complete resistance to Cereal Yellow Dwarf Virus (CYDV) and significant but partial resistance to Barley Yellow Dwarf Virus (BYDV). It was discovered that the resistance to CYDV is due to a block in virus movement and alters the feeding behavior of the aphid vector. Additional research is underway to understand what is preventing the movement of CYDV within the wheat plant. Determining the mechanism of this resistance will allow us to identify methods for improving resistance to Barley Cereal Yellow Dwarf Virus (BYDV) and other plant viruses. It was proved that BYDV replicates much faster in wheat than CYDV, that

replication of YDV begins within hours in oat compared to days in wheat, and that substantially more BYDV and CYDV accumulates in oat compared to wheat.

Impact: These cultivars are the first lines released in the United States which have very high levels of YDV resistance, excellent agronomic traits and constitute the first and, thus far, only instance of successfully breeding YDV resistance into elite wheat varieties in the United States. National and international wheat breeding programs have submitted many requests for this material. These lines are now the basis of YDV resistance for all wheat breeders within the United States and in many places across the world. Following our finding that CYDV wheatgrass-derived resistance is due to a block in virus movement, we proposed the first and only mechanism model of YDV resistance. Knowledge of the resistance mechanism and the biology of YDV were essential to achieving effective resistance and implement screening strategies for resistance in the breeding program. Yellow dwarf disease is caused by single or mixed infections of a complex of viruses, yet prior to this work the rate of viral replication and how and when wheat and oat plants respond to virus infection were not known. These data are beginning to explain why BYDV is a more serious viral pathogen than CYDV in wheat and why oat is a more YDV-susceptible host. By linking these data with BYDV and CYDV resistance and generalized plant defense responses, significant progress is being made in correlating changes in gene expression to wheatgrass-derived YDV resistance and YDV susceptibility in wheat and oat. The real-time PCR assay has the potential for supplanting the standard ELISA currently used throughout the world for YDV diagnosis as it is more sensitive and reduces the assay time by 90%. The results from this research have generated improved cultivars and knowledge of resistance pathways that are significant contributions towards the goal of increasing of small grain cereals yields. These data were used for correlating changes in host-encoded gene expression and virus accumulation after inoculation.

Additional Information: The YDV resistant cultivars and germplasm line were products of the Cooperative USDA-ARS/Purdue University Small Grains Breeding Program. During the development of these cultivars, the Purdue University Small Grains Breeder, performed the crosses and field evaluations. ARS performed all aspects of the greenhouse and growth chamber phenotypic testing and all molecular genetic analyses of these lines to define their genetic composition. The resistance mechanism research has also been facilitated by collaborations with non-ARS scientists who provided many of the wheat and oat lines used in this research.

The evaluation of the wheat virus-induced gene silencing system for functional analysis of genes required for disease resistance has been facilitated by collaborations with non-USDA scientists. These collaborators study wheat disease resistance pathways for which the R-gene has been identified and cloned.

Documentation:

Crasta, O., Francki M.G., Bucholtz D.L., Sharma H., Zhang J., Wang R-C., Ohm H.W., Anderson J.M.. 2000. Identification and characterization of wheat-wheatgrass translocation lines and localization of barley yellow dwarf virus resistance. Genome 43:698-706.

Balaji, B, Bucholtz, D.B., Anderson, J.M. 2003. *Barley yellow dwarf virus* and *cereal yellow dwarf virus* quantification by real-time PCR in resistant and susceptible plants. 2003. Phytopathology. 93:1386-1392.

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Wiangjun, H., Anderson J.M. 2004 The basis for *Thinopyrum*-derived resistance to *Cereal yellow dwarf virus*. Phytopathology 94:1102-1106. 2004

Ohm, H.W., J.M. Anderson, H.C. Sharma, L. Ayala, N. Thompson, J.J. Uphaus. 2005 Registration of Yellow Dwarf Viruses Resistant Wheat Germplasm Line P961341. Crop Sci. 45: 805-806. 2005

Citrus Tristeza Virus

Background: Thousands of citrus acres are destroyed each year in Florida because of damage by Citrus Tristeza Virus (CTV). The development of resistant citrus cultivars by conventional breeding or transformation and regeneration techniques requires ten or more years. Over the last few decades, ARS scientists have developed populations that are now yielding elite selections for new rootstocks and scions resistant to CTV and other diseases. Preliminary screening with molecular markers and testing with controlled inoculations are used to select the most promising candidates. New cultivars are officially released after field trials at multiple locations verify outstanding performance under commercial conditions.

Accomplishment: One new citrus rootstock cultivar that is highly resistant to CTV was developed by ARS scientists and released in 2001. This rootstock, named US-812, was created by conventional breeding among citrus relatives and has proven exceptionally productive of high quality fruit at many locations in Florida. Other new rootstock and scion cultivars with resistance to CTV and other diseases are now undergoing the final stages of testing before release.

Impact: US-812 rootstock has already been used to replant commercial citrus trees dying from CTV and other problems on several hundred acres of Florida citrus. Demand for trees on this rootstock continues to exceed the ability of the citrus nursery industry to

produce them. Three other new ARS rootstocks that combine CTV resistance with tolerance to other pathogens are eagerly awaited by Florida citrus growers.

Additional Information: Funding from the Florida Citrus Industry to support the development of new citrus rootstock and scion cultivars has been provided since 1998 through Trust Fund Agreements. This funding is contributing to the project by providing additional personnel, supplies, and equipment to create, select, and test new rootstock and scion cultivars. A BARD funded project has also facilitated the study of citrus fruit pathogen resistance.

Documentation:

Bowman, K. D. 2001. Notice to fruit growers and nurserymen relative to the naming and release of the US-812 citrus rootstock. Approved by Administrator on 11 May 2001.

Bowman, K. D. and S. M. Garnsey. 2001. A comparison of five sour orange rootstocks and their response to citrus tristeza virus. Proc. Fla. State Hort. Soc. 114:73-77.

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Cereal Rusts

Background: Rusts, especially stripe rust**,** are the most important diseases in the western United States and stripe rust has become increasingly important in the Great Plains. The goal of this project is to reduce losses in yield and quality caused by rusts, especially stripe rust. This project is coordinated with state, regional, national, and international research programs on control of wheat and barley rusts. The research provides an essential service to wheat and barley breeders, cereal pathologists, extension and advisory personnel, and wheat and barley growers. Both basic and applied research has been conducted to accomplish the following objectives: 1) determine factors influencing epidemic development, population structures, and host-pathogen interactions for rusts; improve rust prediction and control, 2) evaluate germplasm and breeding lines of wheat and barley for resistance to rust diseases; and 3) identify new sources of resistance to stripe rust, determine genetics of resistance, develop molecular markers for

mapping and cloning resistance genes, and transferring resistance to commercial cultivars, and combining different genes to obtain durable and superior resistance.

Accomplishment: Every year, rusts and other foliar diseases of wheat and barley were monitored throughout the United States through cooperators. Stripe rust outbreaks were accurately forecasted, especially for the western United States. The disease epidemic information was used to forewarn growers to implement appropriate control measures, which prevented major damage by wheat stripe rust and saved growers millions of dollars every year. Virulences of more than 300 stripe rust samples and frequencies and distributions of races collected throughout the United States were determined and characterized every year. Since 2000, 59 new races of the wheat stripe rust pathogen and 19 new races of the barley stripe rust pathogen have been identified.

Every year, more than 20,000 wheat and barley entries were evaluated for resistance to stripe rust in fields and/or greenhouse. The entries included germplasms, regional cultivars, breeding lines, and genetic populations from breeders and the USDA-ARS National Small Grains Collection Center. The entries were also evaluated for resistance to leaf rust, stem rust, powdery mildew, barley scald, and physiological leaf spot in fields when these diseases occurred naturally. Resistant germplasms and breeding lines were identified and characterized. The information was provided to breeders for developing resistant cultivars.

ARS tested efficacy and determined rates, timing of application, and impact on yield of several fungicides to control stripe rust. The information is used by chemical companies to register new fungicides.

Impact: This project is the leading program on stripe rust research in the United States and the world. The program plays an essential role in monitoring and predicating the disease throughout the nation. This information is provided to breeders and pathologists throughout the country each year for guiding the development of resistant cultivars, and to growers to aid selection of cultivars with effective resistance to prevent major losses. In the last five years, more than 10 wheat cultivars and 3 barley cultivars were released from the USDA-ARS and Washington State University breeding programs and many from other states, resulted from the collaborations with breeders. The information on fungicide efficacy and cultivar responses and the implementation of appropriate control measures using the information from this project saves growers millions of dollars every year. The concept of durable HTAP resistance has been accepted by most breeders and pathologists and is used in the development of resistant cultivars for sustainable control of stripe rust. Breeders throughout the country have used resistance sources, genes, and markers for the genes identified and developed in this project. The resistance gene analog polymorphism technique developed in this project has been widely used by many laboratories throughout the world in developing molecular markers and cloning genes for resistance to many diseases. The wheat and stripe rust BAC and full-length cDNA libraries are noteworthy contributions to the wheat research community for studying the wheat genome and its functional interaction with the stripe rust fungus, as well as numerous other systems.

Additional Information: Outside (non-USDA) funding has been obtained to accomplish some phases of the research. The sources of the outside funding include Washington Wheat Commission, Washington Barley Commission, Idaho Wheat Commission, U.S. Barley Genome Project, and chemical companies.

Documentation:

Shi, Z.X., Chen, X.M., Line, R.F., Leung, H., Wellings, C.R. 2001. Development of resistance gene analog polymorphism markers for the *Yr9* gene resistance to wheat stripe rust. Genome. V. 44 p. 509-516.

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Chen, X.M. 2004. Epidemiology of barley stripe rust and races of *Puccinia striiformis* f. sp. *hordei*: the first decade in the United States. Cereal Rusts and Powdery Mildew Bulletin (www.crpmb.org/) 2004/1029chen.

Multiple Pathogens of Sugarcane

Background: Sugarcane diseases cause yield losses in susceptible cultivars. Resistant cultivars are the most effective method of control and can be developed by using the proper screening programs to identify and eliminate cultivars susceptible cultivars from populations having diverse disease reactions. New sources of resistance need to be identified particularly for sugarcane yellow leaf virus since relatively few parental clones are resistant and the World Collection of Sugarcane and Related Grasses at the Subtropical Horticultural Research Station offer a wide diversity of clones and possibly new sources of resistance.

Accomplishments: Eight sugarcane cultivars that had adequate field resistance to smut, leaf scald, mosaic, rust, ratoon stunt and eye spot were released to the sugarcane growers. These cultivars offer new options for commercial production in Florida. Yield losses were detected due to sugarcane yellow leaf virus in commercial cultivars. Additionally, the presence of sugarcane yellow leaf virus was widespread in clones of the World Collection of Sugarcane and Related Grasses; however, punitive resistant clones were detected of all species of *Saccharum*. Clones of *S. spontaneum* had the lowest incidence

of the virus. Sugarcane populations were characterized for the reaction to ratoon stunt, rust and yellow leaf for use in molecular research to associate markers with resistance.

Impact: The newly released cultivars offer alternate options in Florida for commercial sugarcane production and may increase yields if adopted. The newly released cultivars will be used in the crossing program to produce sugarcane clones for the variety development program. Molecular research to associate disease resistance with markers can proceed using the sugarcane populations characterized for their ratoon stunt, rust and yellow leaf reactions.

Additional Information: Four grants from the Florida Sugar Cane League were received for support of laborers used in the disease screening and pathology program. This support allowed the screening of clones (1,000 to 1,500 clones) for their reaction to ratoon stunt at an earlier stage in the program thereby eliminating susceptible clones before they were placed in replicated yield tests. A grant from the National Plant Germplasm Resource System allowed the screening of the clones in the world Collection of Sugarcane and Related Grasses at the USDA-ARS Subtropical Horticulture Research Station, Miami, Florida to be screened for the presence of sugarcane yellow leaf virus and thus, identify putative resistant clones to the virus.

Documentation:

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Multiple Pathogens of Grape and Apple Rootstocks

Background: Apple growers face the challenge of fireblight disease and apple replant disease. Apple replant disease is a disease complex with fungal, bacterial, and nematode components that damages apple trees planted into sites formerly occupied by apple orchards. Since the best orchard sites are desired for continued production, apple replant disease is a serious problem in many apple growing regions, including Washington and New York. Grape growers face the challenge virulent root-knot nematodes, which feed

on and damage contemporary nematode resistant rootstocks. Since the contemporary nematode resistant rootstocks all owe their nematode resistance to the same allele, identifying novel superior alleles for resistance to virulent nematodes and deploying those superior alleles in improved rootstock varieties is a top priority for grape improvement.

Accomplishment: Four apple rootstock selections have been identified and scheduled for release to growers as improved varieties. These selections are resistant to fireblight disease and provide desirable size control to the scions to which they are grafted. Commercial scale propagation of these selections has begun and soon commercial nursery trees utilizing these rootstocks will be available domestically and abroad. Three more selections, which demonstrate apple replant disease and fireblight resistance as well as providing desirable size control, are in advanced stages of testing and are expected to soon be designated for release to growers as improved varieties. More than 200 grape rootstock selections with resistant to virulent root-knot nematodes have been identified and advanced to the vineyard for additional horticultural testing. The first of these selections will enter grafted vineyard trials in 2005.

Impact: Improved apple and grape rootstocks will reduce the need to apply pesticides to manage soil-borne pests and diseases and will reduce the risk of and damage from fireblight disease in apples. Improved apple and grape rootstocks are a methyl bromide alternative, since they can help substitute for the use of methyl bromide in preplant soil treatment. Growers will realize improved efficiency of production, as the use of rootstocks is cost-effective, representing a one-time cost, but long term benefits. Fireblight resistance reduces the need to apply antibiotics for disease control, representing additional cost savings. Reduced use of pesticides reduces collateral distribution of biologically active chemicals into the environment.

Additional Information: Cooperation with Cornell University scientists was instrumental in the testing, breeding, and release of new apple rootstocks. The apple rootstock breeding program was initiated by Cornell University scientists as a Cornell University project; ARS assumed responsibility for an already scientifically mature program with selections in the product pipeline. Testing in cooperation with Cornell University scientists is instrumental in identifying selections that are suitable for release. Significant outside funding has been received for grape rootstock breeding from the American Vineyard Foundation and the California Table Grape Commission. This outside funding contributes to the development of improved grape rootstock varieties.

Documentation:

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Cousins, P. M. and Walker, M. A. 2001. A technique for screening grape germplasm for resistance to *Meloidogyne incognita*. Plant Disease v. 85. p.1052-1054.

Robinson, T., Aldwinckle, H.S., Fazio G., Holleran T. 2003. The GENEVA series of apple rootstocks from Cornell: performance, disease resistance, and commercialization. Acta Horticulturae v. 622 p.513-520.

Downy Mildew of Cole Crops

Background: Downy mildew is a very destructive fungal disease of cole crops such as broccoli, cauliflower, and collard, and it causes multimillion-dollar losses. Growers typically control the disease with fungicides. However, few products effectively control downy mildew and some may be eliminated from future use. In addition, due to food safety and environmental quality concerns, there is a need to reduce the use of fungicides. Thus, demand for mildew-resistant broccoli varieties is increasing because they will provide the safest effective alternative to fungicides for downy mildew control. A specific objective this project was to develop and characterize downy mildew resistant broccoli germplasm that can be used as parents for producing resistant hybrids that do not require fungicide applications to control this disease. Another goal of this cole crop research was to mark the actual resistance genes at the DNA level for use in marker-assisted selection methods.

Accomplishment: It is essential to identify and characterize genes conferring downy mildew resistance in cole crops so such genes can be effectively bred into commercial varieties and used to prevent damage due to this very destructive disease. A series of studies at the U.S. Vegetable Laboratory confirmed the presence of very high resistance to downy mildew in numerous ARS breeding lines developed by the project, and at least three different genes conferring resistance in these lines were elucidated using conventional and biotechnological methods. In addition, DNA fingerprint markers linked to these resistance genes were identified and specific PCR-based sequence-characterized amplified region (SCAR) markers that identify an important cotyledon stage resistance gene were developed. The project also developed an inbred line of broccoli, designated USVL012, that exhibits a high level of resistance to downy mildew from the transplant stage through maturity and that also has good horticultural characteristics.

Impact: Sources of downy mildew resistance genes, like USVL012, have been provided to public and private cole crop breeders who are using these resources to develop commercial, downy mildew-resistant broccoli hybrids. These resulting hybrids may serve as new broccoli cultivars that will not require the use of fungicides to control downy mildew. DNA sequences of PCR-based markers that identify resistance genes have also been supplied to public and private scientists who are using them to mark resistant individuals in segregating populations and to speed up the resistance breeding process. Ultimately, this research will help to reduce cole crop losses by contributing to a reduction in the negative impact of downy mildew in lowering yields and vegetable quality.

Additional Information: A gift from Syngenta Seed helped to fund a graduate student who identified markers linked to cotyledon stage resistance and developed SCAR markers for this trait.

Documentation:

Farnham, M.W., Wang, M., Thomas, C.E. 2002. A single dominant gene for downy mildew resistance in broccoli. Euphytica. 128:405-407.

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Nematode Resistant Peas

Background: Southernpea (cowpea, blackeye bean) is an important component of the modern American diet, and genetic improvement of this crop is needed to meet demands for increasing production efficiency and for producing greater quantities, higher quality, and more readily available fresh, dry, and processed southernpea products. Root-knot is a severe disease of southernpea that is caused by several species of the root-knot nematode genus *Meloidogyne*. The southern root-knot nematode is estimated to cause 5 to 10% loss in southernpea yields. An estimated 30% of the blackeye bean acreage in California is grown in *Meloidogyne*-infested fields, and 100% of the acreage is considered to be at risk. Several of the leading horticultural-type southernpea cultivars grown in the United States are susceptible to root-knot nematodes. The most economical and environmental friendly method of controlling root-knot nematodes in southernpea is by the development and utilization of resistant cultivars.

Accomplishment: Blackeye-type southernpeas are widely grown by fresh market growers and home gardeners throughout the southeastern United States. About 15 years ago, ARS researchers, initiated efforts to develop an improved, root-knot nematode resistant blackeye-type cultivar that is particularly suited for use by fresh market growers and home gardeners. The new cultivar, named Charleston Blackeye, has an upright plant habit, and is more resistant to lodging than Bettergro Blackeye. Charleston Blackeye is homozygous for the *Rk* gene that conditions a high level of resistance to root-knot nematodes. A typical fresh-shell stage pod is an attractive yellow color, long (24 cm), straight to slightly curved, exhibits only slight constrictions between peas, and contains 15 peas. Fresh peas exhibit a primary cream color, have a kidney shape and a small black

eye, and weight 36 g per 100 peas. Dry peas have a rough seed coat.

Impact: A request for approval to release Charleston Blackeye was made on November 24, 2004; the release notice was approved on January 31, 2005. The approved cultivar release notice distribution list included a total of 177 seed companies and research/extension personnel. Since seed requests have not yet been filled, it is too early to judge impact. Charleston Blackeye is recommended for use by home gardeners for the production of fresh-shell, blackeye-type peas. Charleston Blackeye is also recommended for trial by market gardeners to produce attractive fresh-shell stage pods and fresh-shell peas for sale in farmers' markets. The new cultivar is especially adapted for production in the southeastern United States.

Documentation:

Fery, R.L., Thies, J.A. 2000. Inheritance of resistance to the peanut root-knot nematode in *Capsicum chinense*. Journal of the American Society for Horticultural Science 125(5):615-618.

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Multiple Pathogens of Sugarcane II

Background: The efficiency of sugarcane production in Louisiana is limited by diseases. The primary goal of this research is to identify commercial-type sugarcane varieties and plants of wild sugarcane relatives that are resistant to the major diseases to use as parents for the next generation of varieties. Genetic markers linked to genes for disease resistance are being sought which would allow scientists to identify plants resistant in a laboratory test rather than having to conduct large-scale field tests that require the exposure of the plants to disease agents. To ensure that sugarcane varieties are resistant to the populations of pathogens found in Louisiana, the distribution of races, strains, or other genetically different groups among the populations of disease agents are investigated.

Accomplishment: New varieties of sugarcane have been released with improved disease resistance, particularly resistance to mosaic, leaf scald, and smut. The development and adaptation of nucleic acid-based diagnostic protocols have led to the discovery of new pathogens and genetic changes among endemic pathogens in sugarcane grown in Louisiana. The protocols were critical to determining the effects of sugarcane yellow leaf virus (SCYLV), a pathogen first reported in Louisiana in 1996, on sugar yield and quality of extracted juice. Sugar yields of plants infected with SCYLV were reduced in a variety of sugarcane that is no longer grown in Louisiana, but not in two currently recommended varieties. In all varieties tested, however, juice quality was affected by SCYLV as a result of increased starch content in leaves and immature portions of the stalk. An increase in starch may affect milling efficiency. Nucleic acid-based protocols were also used to show that new strains of sorghum mosaic virus (SrMV), the cause sugarcane mosaic in Louisiana, are present and that the incidence of a minor strain has become predominant replacing a strain that was predominant for over 30 years.

Impact: The new varieties of sugarcane offer growers well-adapted, high-yielding varieties that have high levels of disease resistance as alternatives to the variety LCP 85-384 that occupies over 90% of the sugarcane fields in Louisiana. Although yield loss caused by SCYLV infection was not demonstrated in LCP 85-384, research at other locations has shown that SCYLV infection can cause significant yield loss in commercial varieties, and severe symptoms have been observed in non-commercial varieties in Louisiana. Consequently, a project to screen new varieties for SCYLV resistance has been established. The varieties in the yield tests were susceptible to natural infection of the virus. To prevent the build up of SCYLV in the Louisiana sugarcane industry, the seed cane certification standards now include a test for the presence of SCYLV in fields of cane to be harvested and sold as seed cane. Sugarcane to be used as parents in the breeding program and near-release varieties are now tested for resistance to all known strains of SrMV rather than the predominant strain only. The availability of specific, sensitive nucleic acid-based diagnostic protocols was important in the development of procedures that allow the direct shipment of sugarcane cuttings between Louisiana and Florida without having to spend six months in Quarantine. Because of the favorable conditions for sugarcane flowering at the APHIS Quarantine Facility in Florida, parent varieties are sent annually to Florida for crossing and the resulting seed returned to Louisiana for germination and selection.

Additional Information: Grants were received from the American Sugar Cane League during the years 2000-2004. The primary use of these funds was to provide seasonal labor needed to conduct field experiments. The areas of research supported by this funding included the genetic variability within the population of viruses causing sugarcane mosaic; improved screening techniques for resistance to ratoon stunting disease, leaf scald, and mosaic; and the importance of sugarcane yellow leaf virus.

Documentation:

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Hop Powdery Mildew

Background: Hop powdery mildew is an emerging disease that has the potential to destroy entire hop yards planted to susceptible genotypes. The disease was first observed in Pacific Northwest hop growing regions in 1997 and hop producers were completely unprepared. The first year, growers lost approximately 25% of all hop production due to large scale production of highly popular susceptible varieties. In following years, growers obtained registrations of more effective, but expensive, compounds but needed to apply up to six sprays to obtain adequate control. Furthermore, chemicals that were effective at controlling powdery mildew did not control downy mildew. The significant increases in production costs were not covered by higher prices because these hops are marketed in a highly competitive world market.

Accomplishment: A new genotype, 'Newport', with excellent brewing characteristics that has resistance to both powdery mildew and downy was developed and released.

Impact: The impact of this USDA-ARS germplasm release is not fully realized because breweries are slow to contract for the production of a new hop variety until extensive brewing evaluations have been conducted. U.S. micro-breweries are buying the limited quantities of this variety that are available. Growers producing this variety have reported significant savings in fungicide applications. Additionally, the USDA-ARS hop breeding program has used Newport as the basis for new genetic crosses designed to improve currently favored hop varieties for disease resistance and yield while maintaining the brewing quality of the original hop.

Documentation:

Henning, J., S. Townsend, W. Mahaffee, S. Kenny and A. Haunold. Registration of Newport Hop. Crop Science 2004 v. 44 p. 1018-1019

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Nematodes and TSWV of Peanut

Background: Breeding and genetic research is being conducted to address three major problems in peanut. The first problem is preharvest aflatoxin contamination (PAC). It is being resolved by the development of peanut germplasm with resistance to PAC. The second problem is yield and quality losses caused by the peanut root-knot nematode. This problem is also being resolved by the development of peanut germplasm with resistance to this pathogen. The third problem is a lack of utilization of valuable genes contained in the U.S. germplasm collection of peanut. This problem is being resolved by the development and refinement of a peanut core collection to improve the efficiency of germplasm evaluations.

Accomplishment: Breeding lines have been developed that have relatively low aflatoxin contamination and relatively high yield when grown under late season heat and drought conditions. Varieties with resistance to aflatoxin contamination would be valuable tools in addressing the most serious challenge facing the U.S. peanut industry.

Breeding lines have been developed that have resistance to both the peanut root-knot nematode and tomato spotted wilt virus (TSWV). These two pathogens currently cause annual losses of over $40 million to the peanut crop in the southeastern region.

More efficient methods to identify valuable genes in germplasm collections are needed. A core of the core (mini-core) collection for the U.S. germplasm collection of peanut was developed. Data on disease resistances were then used to retrospectively determine how effective the use of a core of the core collection would have been in identifying sources of resistance in the core collection. Results indicated that the mini-core can be used to improve the efficiency of identifying desirable traits in the core collection and in the entire collection. This mini-core approach should be particularly useful for traits that are difficult and/or expensive to measure.

Impact: The American Peanut Council, representing all segments of the U.S. peanut industry, has identified PAC as the most serious challenge facing the industry. Progress in developing resistant cultivars would represent a major advance for the U.S. peanut industry.

The size of germplasm collections for all crops increases over time, which in turn increases the costs of collection maintenance and the evaluation expenses needed to identify particular genes. The testing and refinement of the core collection theory is expected to reduce these expenses for all crop species.

Documentation:

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Holbrook, C. C., Stephenson, M. G., Johnson, A. W. 2000. Level and geographical distribution of resistance to Meloidogyne arenaria in the U.S. peanut germplasm collection. Crop Sci. 40:1168-1171.

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Wheat Powdery Mildew

Background: Diseases are responsible for the loss of crop yield, end-use quality, and genetic diversity in cereal crops. Sources of resistance need to be identified and incorporated into adapted genotypes to combat polymorphic pathogen populations. The genetic basis of disease resistance in cereals needs to be widened to prevent genetic vulnerability. Molecular and genomic techniques need to be utilized to better select disease resistance genes in segregating plant populations and homozygous lines, as well as to identify virulence and population dynamics of pathogens. Disease management strategies that incorporate host genetic diversity are needed to extend the useful life of resistance genes. Molecular marker-assisted selection and identification of disease resistance genes are needed in cereal genotypes adapted to the southeastern United States. The gap between discovery of molecular genetic information and the use of that information in practical cereal improvement needs to be bridged.

Accomplishment: New sources of seedling and adult-plant resistance to wheat powdery mildew (caused by *Blumaria graminis* f. sp. *tritici*) need to be identified and introgressed into adapted genotypes to combat the highly variable pathogen population. This research identified new sources of resistance in ancestral relatives of wheat, crossed and backcrossed the new resistances into 45 adapted germplasm lines and cultivars of both soft and hard winter wheat, and selected homozygous resistant lines from the resulting segregating populations. These lines serve as new sources of powdery mildew resistance in wheat for direct selection or to be used as parental material by wheat breeders in the development of new cultivars.

Impact: These novel sources of disease resistance are already in adapted genotypes, thus direct selection can be practiced for local adaptation, or the lines can be used as parents in crosses without losing grain yield potential or superior end-use quality. Moreover, these lines each have a different gene for resistance to powdery mildew, which are effective in either eliminating initial inoculum (seedling resistance) or in mitigating disease increase (adult-plant resistance). In addition, the lines were selected for superior end-use quality (both hard and soft wheat quality), thereby eliminating the deleterious quality effects sometimes associated with crosses between adapted wheat and its wild progenitors.

Additional Information: Collaborations for field testing the lines and verifying the effectiveness of the resistances were established with the University of Georgia, VPI, the University of Maryland, and the University of Kentucky.

Documentation:

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Srnic, G., Murphy, J. P., Lyerly, J. H., Leath, S., and Marshall, D. 2005. Inheritance and chromosomal assignment of powdery mildew resistance genes in two winter wheat germplasm lines. Crop Science (in press).

Fungal Pathogens of Bean

Background (1): *Sclerotinia* white mold is the most important disease limiting common bean production in the United States. Genetic resistance as a component of integrated management strategies for control of white mold in bean is generally lacking because resistance is extremely complex and difficult to breed for. In addition, detection of resistance genes via pathogen screening is unpredictable because adequate and uniform disease epidemics rarely occur, and greenhouse screening does not always correlate well with field reaction. Thus, development of white mold resistant cultivars is an arduous, laborious, and time-consuming enterprise.

Accomplishment (1): Three genes from two exotic sources that combat white mold disease of common bean were identified, characterized, and mapped. Linked markers have been refined to facilitate marker-assisted selection of the genes across laboratories.

Impact (1): Marker-assisted selection for the three genes identified and tagged was validated in a pinto bean background and has been used to develop enhanced germplasm lines USPT-WM-1 and USPT-WM-2 with improved resistance to white mold. Breeders will be able to use these germplasm and markers to develop resistant cultivars in 4 to 5 years versus 8 to 10 years time using only traditional breeding methods. The availability of white mold resistant cultivars will increase the effectiveness of integrated disease control strategies saving growers millions of dollars via improved yields and reduced input (fungicide) costs.

Background (2): Anthracnose, a devastating seed-borne disease of beans, was observed in pinto bean fields in North Dakota and Minnesota in 2001, placing 350,000 acres of susceptible pinto bean production at risk. Genetic resistance provides the best means of combating this emerging disease problem, however pinto beans with resistance are lacking.

Accomplishment (2): USDA-ARS led a team of researchers from Michigan State University, North Dakota State University, and University of Idaho in the rapid development of an anthracnose resistant pinto bean germplasm USPT-ANT-1. Marker-assisted backcrossing using a DNA marker tightly linked with the anthracnose resistance gene *Co-4*^{2} was used to rapidly introgress the gene into the highly susceptible pinto bean background.

Impact (2): The benefit of USPT-ANT-1 is that it possesses the *Co-4*^{2} gene, which confers resistance to all known North American strains of *Colletrotrichum lindemuthianum* that causes anthracnose. USPT-ANT-1 also possesses resistance to bean rust and bean common mosaic virus, which are major bean diseases in the Midwest and seed production region of the Northwest, respectively. This line will be most useful for incorporating resistance to anthracnose primarily in the pinto bean market class, but also in the medium-seeded great northern, pink, and small red market classes as well. All the U.S. dry bean breeders, public and private, have already requested and received seed of USPT-ANT-1 germplasm line for testing and for transferring resistance into pinto bean cultivars. The USPT-ANT-1 germplasm will be critical to combating future outbreaks of anthracnose disease in the Upper Midwest. This research also documents the utility and power of marker-assisted selection for rapid deployment of a disease resistance gene to combat an emerging disease problem.

Background (3): Bean golden yellow mosaic is a devastating viral disease of beans in tropical and subtropical Latin America and Florida. In fact it is the number one disease problem of those regions. Genetically resistant cultivars combined with restrictions on planting date are used to control this disease. Genetic control is difficult to attain because it requires combining different resistance genes from multiple sources, and few sources of resistance exist. New sources of durable resistance are needed to expedite cultivar development.

Accomplishment (3): Two new genes conditioning a high level of resistance to bean golden yellow mosaic virus were discovered and successfully transferred from scarlet runner bean (a related species) to three dry edible bean germplasm releases: PR0157-4-1 (small white), PR9771-3-2 (small red), and PR0247-49 (shiny small black). ARS scientists performed the original interspecific hybridization and conducted disease screening and pedigree selection for the first five generations. University of Puerto Rico scientists and graduate students conducted the inheritance study, the final cross, and disease screening and pedigree selection leading to the released germplasm lines. Both researcher groups contributed to the DNA marker assays.

Impact (3): The benefit of PR0157-4-1 (small white), PR9771-3-2 (small red), and PR0247-49 (shiny small black) is that they possess novel genes that condition a high level of resistance to bean golden yellow mosaic virus disease. Other laboratories in Brazil, Colombia, and Florida have been pursuing resistance from scarlet runner bean for a long time, but this is first research to fully characterize the resistance and move it into adapted lines that breeders can readily use in development of new cultivars. The lines have already been requested by breeders in Brazil and Colombia, and will be freely disseminated throughout the rest of Latin America and the United States. These lines represent the first novel germplasm in more than 10 years that will lead to significant improvements in the level and durability of resistance to bean golden yellow mosaic virus.

Additional Information: Collaborations with University (North Dakota State University, University of Idaho, Michigan State University, Oregon State University, Washington State University, University of Puerto Rico, University of California-Davis, University of Florida), Private Seed Company (Syngenta, Harris Moran) and International (South Africa, Tanzania, Malawi, and CIAT-Colombia). Scientists contributed significantly the above and other research conducted by the project since 2000. Outside funding from US-AID Bean Cowpea CRSP, ARS Sclerotinia Initiative, FAS, PCGC, and Cool Season Legumes – Special Grants Program (U. Idaho) contributed to the research.

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Fungal Pathogens of Sunflower

Accomplishment: ARS initiated two Sclerotinia head rot nurseries with automated mist irrigation in North Dakota and five stalk rot nurseries in North Dakota, Minnesota and South Dakota. This research succeeded in producing Sclerotinia ascospores to induce

head rot, and developed an inocluation method and tractor-drawn implement to inoculate thousands of rows to initiate stalk rot. These nurseries were used to evaluate both USDA sunflower breeding material as well as characterizing the disease resistance of commercial hybrids. The net result of these efforts has been the release of the first public sunflower germplasm with resistance to Sclerotinia stalk rot, and the pending release of the first sunflower germplasm with high levels of resistance to both Sclerotinia head rot and stalk rot. The stalk rot inoculation method, coupled with mechanized inoculation, has allowed us to test 75 commercial hybrids at multiple sites, which will generate data in 2005 for publication in extension publications. The inoculation method, while a modification of previous techniques, is simplified and more efficient, and will allow public and private researchers to test large numbers of entries for resistance to what is the leading sunflower disease around the world. ARS also developed a diagnostic test that will detect the presence of the sunflower downy mildew pathogen, *Plasmopara halstedii*, in soil samples. This procedure allows the detection of the pathogen well in advance of symptom appearance in field situation. In analyzing several hundred soil samples collected in eight Midwestern states, it was documented that the fungus is present in 60 to 80% of fields, in contrast to ~30% incidence during actual disease surveys.

Additional Information: ARS efforts in developing sunflower germplasm with resistance to Sclerotinia head rot and stalk rot are largely internal, but would not be possible without the involvement of personnel of North Dakota State University (specifically the Carrington, North Dakota) and South Dakota State University. The National Sunflower Association provided funding for the establishment of the first automated mist nursery at Carrington. The majority of support for the Sclerotinia accomplishments has come from competitive grants obtained from the USDA Sclerotinia Initiative.

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Fungal Blight of Chickpea

Background: Ascochyta blight caused by *Ascochyta rabiei* is the most destructive disease of chickpea in the United States and all major chickpea production regions. There is no immunity to this disease in cultivated chickpea and its close relatives. Resistance is a quantitative trait. Furthermore, the pathogen shows considerable genetic variability in pathogenicity. Up to seven systems have been proposed to classify the pathogenic variation, but each system used a different set of differentials, making it difficult to compare results among different studies. A standardized procedure to classify the pathogenic variation is needed in order to understand the pathogen variability and to reliably evaluate inheritance of host resistance.

Accomplishment: A mini-dome technique was developed for consistent pathogenicity assay of *Ascochyta rabiei*. The technique was used to evaluate pathogenic variations among isolates of the pathogen and to evaluate relative resistance of chickpea germplasm lines to the disease. The results have led to classification of the pathogen into two pathotypes. Chickpea responses to the two pathotypes show distinct distribution patterns (bimodal response to pathotype I and quantitative response to pathotype II). Chickpea resistance to pathotype I was mapped to a major genetic locus and resistance to pathotype II was mapped to two quantitative trait loci. In addition, application of the two-pathotype system determined that pathotype II currently dominates the pathogen population in the Unite States, demonstrated the improved resistance to pathotype II in the recently released cultivar 'Sierra', and identified germplasm lines that are highly resistant to pathotype II of *A. rabiei*.

Impact: Standardized pathogenicity assay with defined differentials will facilitate comparison of research results and communications among scientists and practitioners of chickpea production. Clearly defined pathotypes will help identify genetic loci conditioning resistance to the pathogen, and allow increased precision in selection for resistance, and allow cloning and characterization of resistance genes. Demonstration of the improved resistance to Ascochyta blight in recently released cultivar will reduce fungicide applications, reduce production cost and environment pollution, and improve farmers' profitability. Identification of resistance sources will allow further improvement in chickpea resistance to Ascochyta blight as part of our long-term research objective with the ultimate goal to enhance U.S. farmers' competitiveness in the world market.

Additional Information: Partial funding from the USA Dry Pea and Lentil Council has facilitated the accomplishments.

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Phytophthora of Potato

Problem being addressed: The introduction in the early 1990's of aggressive, metalaxyl - resistant genotypes of *Phytophthora infestans* has changed production practices and costs for the U.S. potato industry. The control of these newer genotypes requires frequent applications of more costly (relative to metalaxyl) fungicides, thereby increasing production costs and reducing growers' profit margins. Even under a strict regime of fungicide applications with apparent control of late blight, late season infection can occur with subsequent infection of tubers. Infected tubers have reduced quality and allow the entry of other storage pathogens with the subsequent decay of tubers in storage.

Accomplishment: The potato variety, 'Defender', was released in 2004. This long, white-skinned, processing variety is notable for having resistance to both foliar and tuber infection by *Phytophthora infestans*. In late blight field trials, Defender displays AUDPC values that are 1/4 as large as those of commonly grown susceptible varieties, indicative of it foliar resistance. In controlled laboratory tests for tuber blight resistance, Defender averaged 3% infected tuber tissue. This is in comparisons with industry standard varieties, Ranger Russet and Russet Burbank, which averaged 52% and 16% infected tuber tissues, respectively in the same evaluations.

Impact: Defender's resistance to late blight (both foliar and tuber) slows disease progression, allowing growers to extend the spray interval between fungicide applications thereby reducing production costs. Profits also would be realized through a reduction in storage losses due to tuber blight.

Additional Information: This project is a partner in the Northwest Potato Variety Development program (a.k.a., Tri-State Potato Variety Development program). Collaborations involving agronomic and disease resistance evaluations of advanced material from our breeding program by personnel at the Idaho, Oregon, and Washington

land-grant universities provides critical information in the decision to release a selection as a variety.

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Multiple Pathogens of Sugarbeet

Background: Sugarbeet is the source of more than half the domestic sugar (sucrose) produced in the United States and is the most profitable crop in rotations in many growing areas. Through the years, advances have been made in sugarbeet genetics to provide improved sugar yields by incorporation of disease resistance and better growth characteristics. Nonetheless, diseases remain an important source of crop and sugar losses throughout the United States and the international community. Additionally, there is growing awareness that serious environmental costs and problems can result from the use of chemical pesticides to control pests and diseases. Thus, it is essential to continue to develop and improve disease resistant germplasm, biocontrol agents, and other means to help maintain a healthy environment and sustainable production system while helping growers remain efficient and competitive in the world market.

Accomplishment: Cercospora leaf spot (CLS) (caused by the fungus *Cercospora beticola* Sacc.) is one of the most widespread diseases of sugarbeet and is a serious problem in many sugar beet production areas throughout the United States. Rhizoctonia root rot (caused by the fungus *Rhizoctonia solani* Kühn) continues to be a problem in most sugar beet-growing areas in the United States, and is a growing problem world wide. Rhizomania caused by *Beet necrotic yellow vein virus* is now confirmed in all

sugarbeet growing areas of the United States. The following germplasms have been released since 2000: FC712(4X) – tetraploid pollinator resistant to *Rhizoctonia solani* and moderately resistant to *Cercospora beticola*; FC724 – monogerm O-type resistant to *R. solani* and moderately resistant to *C. beticola*; FC710(4X) –tetraploid pollinator resistant to *R. solani* and moderately resistant to *C. beticola*; FC201 – released with R. T. Lewellen (USDA-ARS, Salinas, California) – a monogerm O-type resistant to rhizomania, moderately resistant to *C. beticola*, *Aphanomyces* and the *Beet curly top virus*, with some resistance to *R. solani* and sugar beet root aphid; FC301 released with R. T. Lewellen (USDA-ARS, Salinas, California) – monogerm O-type resistant to rhizomania and *C. beticola*, moderately resistant to Aphanomyces and *beet curly top virus*; FC720 – a monogerm O-type resistant to *R. solani* and moderately resistant to *C. beticola;* FC722 – a monogerm O-type resistant to *R. solani* and moderately resistant to *C. beticola;* FC722CMS – a monogerm CMS equivalent to FC722 resistant to *R. solani* and moderately resistant to *C. beticola.*

Additional Information: This research was done in collaboration with the Beet Sugar Development Foundation (BSDF), which is a non-profit organization of Beet Sugar Processing Companies and Sugarbeet Seed Related Companies. The BSDF is dedicated to the advancement of sugarbeet production and beet sugar processing through science based research and leading educational programs. The BSDF has a close working relationship with ARS and provides resources to further projects of mutual interest. In this collaborative effort they provided field personnel, support personnel in the laboratory and IT support personnel. The field screening for *Beet curly top virus* was accomplished in the BSDF-managed nursery at Kimberly, Idaho.

Impact: These germplasm have been requested by seed company breeders from around the world and are being incorporated into commercial breeding programs. They combine resistance to a number of different diseases that the beet seed industry breeders are vitally interested in. All of the Rhizoctonia resistant commercial hybrids used throughout the world have the germplasm from the Fort Collins program as their source of resistance. A continued release of sugar beet germplasm with enhanced disease resistance, superior agronomic qualities, and increased suitability to sustainable production practices will help U.S. growers.

Documentation:

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Pierce's Disease of Grapes

Background: The development of disease resistant grapes is needed to make U.S. growers more competitive in global markets and to help protect the environment. Sources of resistance to powdery mildew and Pierce's disease have been identified but they have poor quality fruit and viticulture characteristics. Development of large populations and numerous backcross generations are necessary of incorporate high fruit quality with disease resistance. The use of embryo rescue for incorporating seedlessness, as well as greenhouse screening and molecular marker assisted selection for resistance, makes conventional breeding quicker and more efficient.

Accomplishments: Grape seedling populations with greatly improved table and raisin grape fruit quality have been developed. Greenhouse screening for powdery mildew resistance has been developed to improve breeding efficiency and verify resistance of individuals identified as resistant in the field. Molecular markers have been identified and verified for selecting Pierce's disease resistant individuals.

Impact: Grape varieties resistant to powdery mildew will greatly reduce or eliminate the need for fungicides sprays for its control. Pierce's disease resistant grapes will increase vineyard life and productiveness as incidence of the disease will be reduced or eliminated. Resistant varieties will also eliminate the concern about vectors for *Xylella* because the plants will be resistant to Pierce's disease. When resistant varieties with the same quality as existing vinifera table and raisin grapes are developed, they will be patented, licensed and provided to growers for production.

Additional Information: The development and evaluation of numerous populations of seedless table and raisin grapes with resistance to powdery mildew and Pierce's disease was made possible by collaboration with the California Table Grape Commission, the California Raisin Marketing Board, California Department of Food and Agriculture, California Competitive Grants Program in Viticulture and Enology and the University of California, Davis, California. The California Table Grape Commission, California Raisin Marketing Board, California Department of Food and Agriculture and California Competitive Grants Program for Viticulture and Enology have funded additional RSA and supplies necessary to produce, grow and evaluate seedling populations and advanced selections.

Cooperation with the University of California has brought together our outstanding table and raisin grape varieties and selections and seedless breeding techniques with his greenhouse screen and development of molecular marker to identify resistant individuals.

This has made possible the development and identification of Pierce's disease resistant individuals with improved fruit quality.

Yearly reports to the California Table Grape Commission, California Raisin Marketing Board, and California Department of Food and Agriculture have been made for both powdery mildew and Pierce's disease resistant variety development work. The seedling populations and advanced selections developed to date are the basis for identifying and verifying molecular markers associated with Pierce's disease. Molecular markers along with greenhouse screening have allowed the development of the BC2 generation within 5 years as the program was first started in 2000.

Advanced table and raisin selections with powdery mildew have been made from eight sources of resistance. They are the core material for development of powdery mildew resistant grape varieties.

Fungal Pathogens of Peanut

Background: Profitable peanut production requires substantial chemical input to manage fungal diseases. Therefore, the development of genetic resistance by conventional means as well as by genetic engineering reduces the reliance on chemical management, and provides sustainable long-term strategies for reducing cost of peanut production. Also, improving the stability of the peanut oil to extend the shelf life of peanut products is a recent concern of the peanut industry in the United States. Reliable methods to quantify the reaction of peanut germplasm, breeding material, and transgenic lines to various fungal organisms under greenhouse and field conditions to facilitate the development of resistant varieties are being developed. Resistance genes exist in the peanut genetic makeup; however, it is desirable to enhance the genetic diversity of the peanut plant by introducing new genes via the deployment of genetic engineering technology to boost disease resistance. Also genes will be introduced to increase oleic acid content for improving shelf life of peanut products.

Accomplishment: Several transgenic peanut lines were developed with enhanced glucanase and chitinase activity. These lines exhibited excellent level of resistance to Sclerotinia blight under field conditions. Also, three peanut cultivars (Tamrun OL 01, Tamrun OL 02, and Olin) with high oleic acid content and acceptable resistance to Sclerotinia blight were released through a cooperative effort between ARS, Texas A & M University and Oklahoma State University.

Impact: Use of disease resistance cultivars by peanut growers will result to lowering production costs by reducing their reliance on fungicides. Lowering cost of production to growers improve their competitiveness in the marketplace. Transgenic peanut research will positively impact and facilitate breeding efforts to develop disease resistant cultivars.

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Discovery Area 2: Molecular Markers, Mapping of Resistance Genes, and Genome Analyses

Background: The genetic manipulation of genes for disease resistance by plant breeders has been employed as a major method to control plant disease since the turn of the century. However, breeders are in need of additional and robust genetics for crop protection. Identification and mapping of genes determining the events in pathogen defense will enhance our understanding of key cellular proteins and processes functioning to mediate resistance and susceptibility. These studies will also provide an understanding of how a limited number of structural classes of resistance proteins enable plants to cope with the potentially large array of diverse pathogens. The discovery of linked molecular markers will facilitate the incorporation of the key genes into new varieties and germplasm, e.g., via marker assisted selection.

Broad Spectrum Resistance Mechanisms

Background: Plants fight infection by viruses, bacteria, fungi, and nematodes by initiating disease resistance responses controlled by resistance (*R*) genes. In tobacco and other *Solanaceae* species, the tobacco *N* gene confers resistance to tobacco mosaic virus (TMV) by triggering a signaling pathway leading to the induction of standard defense and resistance responses. Although our understanding of resistance genes has advanced, many important questions remain unresolved. The identities and functions of the proteins that interact to confer resistance are just beginning to be understood. In practical terms, the identity of genes determining the events in pathogen defense will lend to an understanding of key cellular proteins and processes functioning to mediate resistance and susceptibility. These studies will also help to understand how a limited number of structural classes of R proteins that cope with the potentially large array diverse of pathogens encountered by plants.

Accomplishment: ARS developed and employed a mutant screen to identify a mutant tomato line *sun1-1* (suppressor of N) that is defective in *N*-mediated resistance. Expression of pathogenesis-related *(PR)* genes, a signature of systemic acquired

resistance (SAR), are dramatically suppressed in *sun1-1* plants, while application of exogenous salicylic acid restores *PR* gene expression, indicating that the *SUN1* gene is involved in the SAR pathway upstream of salicylic acid. The *sun1-1* mutation also impairs other resistance (*R*) gene pathways, specifically *Bs4*, *I1*, and *Ve*. Thus, *SUN1* is required for multiple classes of *R* genes, including both TIR (for Drosophila Toll and mammalian Toll-Like and Interleukin-1 receptors) and non-TIR subclasses. sun1-1 plants also exhibit enhanced susceptibility to TMV (even relative to sun-*N* containing plants), as well as to bacterial pathogens. *sun1-1* enhanced pathogen susceptibility provides evidence that *R* gene-mediated pathways and general resistance pathways intersect or overlap. Cloning efforts have revealed *sun1* is a homolog of a gene require for normal resistance responses in Arabidopsis, EDS1, a putative lipase. Work is now focusing on the mechanistic role of EDS1 in the plant, by identifying its subcellular localization, characterizing its lipase activity, and identifying interacting proteins.

Impact: The results of the research on R gene function will be used to facilitate development and application of novel strategies for enhanced plant resistance to pathogens for development of disease resistant crops. Unraveling the molecular basis of disease resistance pathways has laid a foundation for the rational design of crop protection strategies.

The genetic manipulation of genes for disease resistance by plant breeders has been employed as a major method to control plant disease since the turn of the century. However, breeders are in need of additional and robust genetics for crop protection. The recent molecular cloning of several plant genes that confer disease resistance to a diverse range of pathogens has revealed that the encoded proteins have several features in common. These findings suggest that plants may have evolved common signal transduction mechanisms for expressing disease resistance to a wide range of unrelated pathogens. The characterization of the molecular signals involved in pathogen recognition and the elucidation of the molecular events specifying the expression of resistance may lead to the development of novel strategies for disease control. support development of phyto-sensors for detection of pathogens that threaten the quality and safety of food and human health.

Additional Information: Funding was received from NSF Plant Genome Research Program from 1999-2002 (DBI99866) and from 2002-2007 (DBI027856) for a collaborative research project with ARS and non-ARS scientists. Co-PIs are TIGR; ARS, Prosser, Washington; Cornell University; University of Wisconsin, Madison; and the University of Minnesota. Collaborators: Sainsbury Laboratory, UK; Universidad Nacional de Rosario, Ar.

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Cereal Fusarium Resistance

Background: *Gibberella zeae* (anamorph *Fusarium graminearum*) causes Fusarium head blight or scab of wheat and barley. In the last decade, *G. zeae* has caused destructive epidemics on wheat and barley in the United States and Canada with cumulative losses estimated at greater than three billion dollars. The goals of this project are to detect new resistance genes, develop molecular markers for resistance genes, and to add to our basic knowledge of the pathogen.

Accomplishment: One of the sources of resistance to Fusarium head blight is the related Chinese cultivars 'Sumai3' and 'Ning7840'. Our previous studies indicated that an important gene for resistance for these varieties resides on chromosome arm 3BS. This result was validated in two new mapping populations with Ning7840. A genetic diversity study revealed that the resistance gene on 3BS is probably widespread in Japanese and Chinese resistant varieties. The Chinese variety 'Wangshuibai' has high and stable resistance to Fusarium head blight. This variety appears to have the same gene on 3BS as Sumai3, but has two or three additional resistance genes. These new resistance genes may provide higher levels of resistance than the 3BS gene alone.

Impact: The validation of the 3BS resistance gene for Fusarium head blight means that marker assisted selection for the 3BS resistance gene from Ning7840 and Sumai3 can be used to increase resistance to Fusarium head blight. The additional genes discovered in Wangshuibai may provide higher levels of resistance than the 3BS gene alone.

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Cereal Rusts Resistance

Background: Rusts, especially stripe rust**,** are the most important diseases in the western United States and stripe rust has become increasingly important in the Great Plains. The goal of this project is to reduce losses in yield and quality caused by rusts, especially stripe rust. This project is coordinated with state, regional, national, and international research programs on control of wheat and barley rusts. The project provides an essential service to wheat and barley breeders, cereal pathologists, extension and advisory personnel, and wheat and barley growers. Both basic and applied research has been conducted to accomplish the following objectives: 1) determine factors influencing epidemic development, population structures, and host-pathogen interactions for rusts; improve rust prediction and control, 2) evaluate germplasm and breeding lines of wheat and barley for resistance to rust diseases; and 3) identify new sources of resistance to stripe rust, determine genetics of resistance, develop molecular markers for mapping and cloning resistance genes, and transferring resistance to commercial cultivars, and combining different genes to obtain durable and superior resistance.

Accomplishment: Every year, rusts and other foliar diseases of wheat and barley were monitored throughout the United States. Stripe rust outbreaks were accurately forecasted, especially for the western United States. The disease epidemic information was used to forewarn growers to implement appropriate control measures, which prevented major damage by wheat stripe rust and saved growers millions of dollars every year. Virulences of more than 300 stripe rust samples and frequencies and distributions of races collected throughout the United States were determined and characterized every year. Since 2000, 59 new races of the wheat stripe rust pathogen and 19 new races of the barley stripe rust pathogen have been identified.

Using a newly-developed technique, non-race specific, high-temperature, adult-plant (HTAP) resistance was demonstrated for the first time to exist in barley cultivars. ARS

identified, characterized, and mapped new genes conditioning resistance in wheat to the barley stripe rust pathogen and resistance in barley to the wheat stripe rust pathogen. ARS also identified more than 20 new genes in barley genotypes conditioning resistance to barley stripe rust. Collaborating with other scientists, ARS identified a new gene from *Triticum diccocoides* for controlling HTAP resistance and helped to transfer the genes into adapted wheat lines. Molecular markers were developed for several genes in wheat and barley for resistance to stripe rusts. Through collaboration, these markers were used to incorporate the genes into commercial cultivars and to pyramid multiple genes into one cultivar for superior resistance.

ARS scientists also constructed a bacterial artificial chromosome (BAC) library of hexaploid wheat and used the library to clone *Yr5*, a wheat gene conditioning stripe rust resistance. A BAC and a full-length cDNA libraries to study the genome of the wheat stripe rust pathogen were constructed. More than 20 genes with clear known functions have been sequenced. This is the first study on functional genomics of the stripe rust fungus.

Impact: The concept of durable HTAP resistance has been accepted by most breeders and pathologists and is used in the development of resistant cultivars for sustainable control of stripe rust. Breeders throughout the country have used resistance sources, genes, and markers for the genes identified and developed in this project. The resistance gene analog polymorphism technique developed in this project has been widely used by many laboratories throughout the world in developing molecular markers and cloning genes for resistance to many diseases. The wheat and stripe rust BAC and full-length cDNA libraries are noteworthy contributions to the wheat research community for studying the wheat genome and its functional interaction with the stripe rust fungus, as well as numerous other systems.

Additional Information: Outside (non-USDA) funding has been obtained to accomplish some phases of the research. The sources of the outside funding include the Washington Wheat Commission, Washington Barley Commission, Idaho Wheat Commission, U.S. Barley Genome Project, and chemical companies.

Documentation:

Shi, Z.X., Chen, X.M., Line, R.F., Leung, H., Wellings, C.R. 2001. Development of resistance gene analog polymorphism markers for the *Yr9* gene resistance to wheat stripe rust. Genome. V. 44 p. 509-516.

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Yan, G.P., Chen, X.M., Line, R.F., Wellings, C.R. 2003. Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. Theoretical and Applied Genetics. V. 106 p. 636-643.

Chen, X.M., Line, R.F. 2003. Identification of genes for resistance to *Puccinia striiformis* f. sp. *hordei* in 18 barley genotypes. Euphytica. V. 129 p. 127-145.

Chen, X.M. 2004. Epidemiology of barley stripe rust and races of *Puccinia striiformis* f. sp. *hordei*: the first decade in the United States. Cereal Rusts and Powdery Mildew Bulletin (www.crpmb.org/) 2004/1029chen.

Cereal Septoria Resistance

Background: The *Septoria tritici* blotch pathogen of wheat, *Mycosphaerella graminicola*, does not infect barley. Instead, barley is host to *Septoria passerinii*, the cause of speckled leaf blotch, which does not infect wheat. However, the evolutionary relationships between these species and among others in the genus *Mycosphaerella* were not known. Progress on understanding non-host resistance depended in part on developing either *S. passerinii* from barley or *M. graminicola* (asexual stage: *S. tritici*) from wheat into a genetic model for species in the genus *Mycosphaerella*, yet very little was known about the evolutionary history or genetics of either species.

Accomplishment: Large-scale phylogenetic analyses were performed of *M. graminicola*, *S. passerinii*, and many other species thought to be related to *Mycosphaerella*, including other grain crop pathogens such as *Cercospora zeae-maydis* and the banana sigatoka pathogen M. *fijiensis*. The results showed that *M. graminicola* and *S. passerinii* were extremely closely related and could serve as good models to analyze non-host resistance interactions in wheat and barley. The analysis also identified a potentially new species of *Septoria* on the wild barley *Hordeum jubatum.* Work as part of a collaborative project with scientists in the Netherlands led to a genetic linkage map for *M. graminicola*, the first for any species in the genus *Mycosphaerella*. The work also lead to cloning of the mating-type genes from *S. passerinii*, which previously had been thought to be asexual, and to the discovery of active transposable elements (mobile pieces of DNA) within *M. graminicola*.

Impact: The large-scale phylogenetic analyses greatly increased our understanding of evolutionary relationships within the genus *Mycosphaerella*, and indicated that new species probably are stimulated to form by the acquisition of new traits. For example, speciation of *M. graminicola* and its relatives was probably triggered by a host shift from a dicot to a grass host. Speciation of *Cercospora* species probably was initiated by acquisition of the ability to produce the phytotoxin cercosporin. The evolutionary relationships published from this study now have been confirmed in other laboratories worldwide. Work on the genetics of *M. graminicola* has now established this species as a model for its genus and for fungi in the order Dothideales. Based on these accomplishments, a proposal to sequence the genome of *M. graminicola* was picked up last year and the sequencing is being done during early 2005. The impact of the genomic sequence will be enormous and will lead to a global surge of new research on this important plant pathogen. Studies analyzing gene expression during non-host resistance responses of barley to the wheat pathogen were performed based on the results of the phylogenetic analysis and are being prepared for publication.

Documentation:

Adhikari, T.B., Yang, X., Cavaletto, J.R., Hu, X., Buechley, G., Ohm, H.W., Shaner, G., Goodwin, S. B. Molecular mapping of *Stb1*, a potentially durable gene for resistance to septoria tritici blotch in wheat. Theoretical and Applied Genetics. 2004. v. 109. p. 944-953.

Dunkle, L.D., Levy, M. Genetic relatedness of African and United States populations of *Cercospora zeae-maydis*. Phytopathology. 2000. v. 90. p. 486-490.

Goodwin, S.B., Waalwijk, C., Kema, G.H.J., Cavaletto, J.R., Zhang, G. Cloning and analysis of the mating-type idiomorphs from the barley pathogen *Septoria passerinii*. Molecular Genetics and Genomics. 2003. v. 269. p. 1-12.

Ray, S., Anderson, J.M., Urmeev, F.I., Goodwin, S.B. Rapid induction of a protein disulfide isomerase and defense-related genes in wheat in response to the hemibiotrophic fungal pathogen *Mycosphaerella graminicola*. Plant Molecular Biology. 2003. v. 53. p. 741-754.

Shim, W.-B., Dunkle, L.D. Identification of genes expressed during cercosporin synthesis in *Cercospora zeae-maydis*. Physiological and Molecular Plant Pathology. 2002. v. 61. p. 237-248.

Cereal Fungal Resistance

Background. Increased acreage of reduced tillage practices in cereal growing regions of the United States has resulted in higher incidence of fungal diseases of wheat and barley. Although low-till practices generally do not increase the incidence of virus diseases, these diseases still result in serious yield losses in cereal crops. If given proper environmental conditions these yield losses can reach 20 to 30% or more for both fungal and viral diseases. Because genetic resistance is the most affordable and effective means of disease control, an attempt should be made to identify resistance in segregating populations, wild relatives, synthetic, and other available germplasm for potential introgression into resistant varieties. Also, because host resistance is a result of inhibited pathogen virulence, it is critical that there be an understanding of virulence to better understand the complete host-parasite interaction.

This ARS project involves characterization of host-pathogen interactions and pathogen biology in both fungal and viral pathogen systems in order to facilitate a better understanding of the fundamental processes resulting in resistance, or susceptibility and disease development. The knowledge gained may lead to the discovery or development of novel and effective control measures for viral diseases of barley and fungal diseases of both barley and wheat.

Accomplishments: The role of the SnTox1 gene (produced by the fungus *Stagonospora nodorum*) in disease has been identified. Previous to our work, SnTox1 had not been reported and therefore neither had its role in disease development. In this study, the

ITMI wheat population to identify QTLs associated with resistance to *S. nodorum* and investigated the role of the toxin in causing disease. This is important because this disease causes a significant amount of damage to the U.S. wheat crop each year and knowledge of the virulence components of the fungus will assist breeders in developing toxin insensitive and therefore more resistant wheat lines.

Genomic regions harboring QTLs for resistance to *Pyrenophora tritici-repentis* race 5 were identified, and the chromosomal location of the gene conditioning sensitivity to Ptr ToxB (produced by the fungus *Pyrenophora tritici-repentis*) was determined. The toxin-sensitivity gene, which were designated Tsc2, mapped to the distal tip of the short arm of chromosome 2B. This gene was responsible for the effects of a major QTL associated with resistance to the race 5 fungus and accounted for 69 percent of the phenotypic variation. Together, the major QTL on 2BS identified by the toxin insensitivity gene (Tsc2) and a QTL on 4AL explained 73 percent of the total phenotypic variation for resistance to P. tritici-repentis race 5. This work is significant because the results of this research indicate that Ptr ToxB is a major virulence factor, and the markers closely linked to Tsc2 and the 4A QTL can be used for introgression of resistance into adapted germplasm.

A major net blotch resistance gene was identified in the Q21861 X SM89010 DH barley population. Net blotch, caused by *Pyrenophora teres*, results in significant losses to barley each year. The entire DH population was inoculated with multiple pathotypes of P. teres and phenotypic analysis showed that the same resistance locus was effective against all pathotypes. AFLPs and SSRs were used to identify markers flanking a single major resistance locus on chromosome 6H. Markers associated with this major resistance gene can be used by barley breeders to introgress this gene into local varieties.

Investigation of host selective toxins (HST) produced by fungal pathogens is necessary to better characterize host-pathogen interactions. In collaboration with Steven Meinhardt of the NDSU Dept. of Biochemistry, detected a HST produced by *Stagonospora nodorum* and determined that it plays a significant role in disease. The HST was partially purified and a host gene for sensitivity to the HST was identified. The identification and characterization of pathogen virulence factors and their role in disease is critical to the development of germplasm containing durable resistance.

Mechanisms of virus pathogenicity remain relatively poorly understood. The determinants of barley stripe mosaic virus (BSMV) pathogenicity to barley were mapped and specific codons in the alpha-a gene of BSMV were identified which determine its pathogenicity to barley and oat. ARS previously had mapped the chromosomal location of a BSMV resistance gene, with the rationale that identification of a marker in close proximity to the resistance gene would be a first step toward the cloning and characterization of the resistance gene. Together, results will provide the foundation for interpreting gene-for-gene interactions between viruses and their hosts, will help elucidate the interactions among viral genes, and will provide a foundation for discovering the mechanisms of viral pathogenicity and host resistance. This in turn will provide for the future design of effective, molecular approaches to disease control.

Impact: Markers identified can be used for introgression of resistance into adapted germplasm, leading to improved yields and quality. Host resistance to fungal diseases may be to the fungi themselves, fungal-produced toxins, or both. Knowledge of the virulence components of pathogenic fungi will assist breeders in developing toxin insensitive and therefore more resistant wheat lines. The identification and characterization of pathogen virulence factors and their role in disease is critical to the development of germplasm containing durable resistance. Generally, this research has resulted in an improvement of our understanding of host-pathogen interactions and the underlying mechanisms involved in the development of disease. Such information is essential in providing the foundation of knowledge needed to facilitate the development of future novel control strategies.

Documentation:

Friesen, T.L., Faris, J.D. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. *Theoretical and Applied Genetics.* v.109 p.464-471.

Haen, K.M., Lu, H., Friesen, T.L, Faris, J.D. 2004. Genomic targeting and high resolution mapping of the *Tsn1* gene in wheat. *Crop Science.* v. 44 p. 951-962.

Liu, Z., Faris, J., Meinhardt, S., Ali, S., Rasmussen, J., Friesen, T. 2004. Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin by *Stagonospora nodorum. Phytopathology* v.94 p.1056-1060.

Liu, Z.H., Friesen, T.L., Meinhardt, S.W., Ali, S., Rasmussen, J.D., Faris, J.D. 2004. Quantitative trait loci analysis and mapping of resistance to *Stagonospora nodorum* leaf blotch in wheat. *Phytopathology* v.94 p.1061-1067.

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Cereal Fungal Resistance II

Background: The foliar diseases tan spot (TS) and Stagonospora nodorum blotch (SNB) caused by the fungal pathogens *Pyrenophora tritici-repentis* and *Stagonospora nodorum*, respectively, cause substantial yield losses in wheat and durum. Few commercial cultivars possess resistance to these diseases. Novel resistance sources need to be identified and incorporated into commercial breeding programs for the introgression of resistance into elite varieties. The genetic characterization of resistance, the determination of chromosomal locations of resistance genes, and the development of molecular markers tightly linked to resistance genes will facilitate breeding efforts and make the development of resistance varieties more efficient.

Accomplishment: Over 200 accessions of durum wheat relatives have been evaluated for resistance to TS and SNB. A number of these have been found to be resistant to both diseases and will be useful for improvement of durum wheat varieties. In collaboration with other ARS scientists, major genes for resistance to SNB and TS have been identified in HRSW mapping populations. ARS showed that genes conditioning insensitivity to proteinaceous toxins produced by the TS and SNB pathogens coincided with major QTLs for resistance to the fungus. The major resistance genes were identified in various populations on chromosomes 1B, 2B, 2D, and 5B. High-throughput markers for marker-assisted selection have been developed, or are under development, for these genes.

Impact: The durum wheat relatives found to harbor resistance to TS and SNB will be useful in breeding programs for introgressing resistance into elite varieties. Knowledge regarding gene action and chromosomal locations of the major genes for resistance to TS and SNB identified in this research will be useful for incorporation of these genes into HRSW elite varieties. The high-throughput molecular markers developed will expedite the transfer of these genes.

Additional Information: North Dakota State University, Department of Chemistry, conducted experiments to identify proteinaceous toxins produced by the TS and SNB pathogens.

Documentation:

Haen, K. M., Lu, H. J., Freisen, T. L., and Faris, J. D. 2004. Genomic targeting and high-resolution mapping of the *Tsn1* gene in wheat. Crop Sci. 44:951-962.

Liu, Z. H., Faris, J. D., Meinhardt, S. W., Ali, S., Rasmussen, J. B., and Friesen, T. L. 2004. Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by *Stagonospora nodorum.* Phytopathology. 94:1056-1060.

Liu, Z. H., Friesen, T. L., Meinhardt, S. W., Ali, S., Rasmussen, J. B., and Faris, J. D. 2004. Quantitative trait loci analysis and mapping of seedling resistance to *Stagonospora nodorum* leaf blotch in wheat. Phytopathology 94: 1061-1067.

Friesen, T. L., and Faris, J. D. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to PtrToxB in wheat. Theor. Appl. Genet. 109: 464-471.

Xu, S. S., Friesen, T. L., and Mujeeb-Kazi, A. 2004. Seedling resistance to tan spot and Stagonospora nodorum blotch in synthetic hexaploid wheats. Crop Sci. 44:2238-2245.

Potato Fungal Resistance

Background: Commercial potato cultivars are susceptible to many foliar and soil-borne pathogens. Resistances to many of these diseases are found in related *Solanum* species. An ARS Breeding Program is developing disease resistant germplasm in two diploid

species closely related to commercial tetraploids and incorporating these disease resistances into the commercial germplasm base via tetraploid x diploid crosses. Molecular markers associated with disease resistance are also being developed for marker assisted selection strategies.

Accomplishments: QTL mapping has located molecular markers linked to resistance to late blight, early blight, and Verticillium wilt in the diploid *Solanum* populations. One QTL occurred in a genomic region where no other QTLs governing late blight resistance have been reported. One QTL associated with early blight resistance was not linked to late maturity. Linkage disequilibrium mapping has located a QTL for resistance to Verticillium wilt and shown that resistance to Verticillium wilt originated from very few ancestors in the tetraploid population. Quantitative inheritance studies conducted at the diploid level showed that resistance to late blight and early blight were highly heritable traits.

Impact: The diploid potato populations have been and continue to be improved for resistance to late blight and early blight. Superior clones from these populations have been utilized to expand the tetraploid germplasm base via 4x-2x crosses. Approximately 10% of the advanced selections undergoing evaluation as potential new potato cultivars in our breeding program now have 25-50% diploid species in their background. Remnant seed of these diploid and tetraploid populations have been furnished to breeding programs in the United States, Morocco, Haiti, and China. Developing and releasing new potato varieties with improved disease resistance is the goal of this program.

Additional Information: Research supported by the USDA-ARS Potato Special Grants Program from 2000-2004. These funds resulted in the publication of # 2, 3, 4, 5 listed below. Publications 2, 4 were the direct result of funding for the National Late Blight Trials. These funds paid for production and distribution of micro-tubers of breeders' advanced selections and new cultivars for late blight field trials at eight cooperative sites.

Collaboration research with Penn State University not funded by the USDA-ARS Potato Special Grants Program resulted in publication #1 listed below. Penn State Univeristy has been involved in the breeding effort to improve the levels of disease resistance in our diploid potato breeding populations.

Documentation:

Christ, B.J., Haynes, K.G. 2001. Inheritance of resistance to early blight disease in a diploid potato population. Plant Breeding. V. 120 p. 169-172.

Haynes, K.G., Christ, B.J., Weingartner, D.P., Douches, D.S., Thill, C.A., Secor, G., Fry, W.E., Lambert, D.H. 2002. Foliar resistance to late blight in potato clones evaluated in national trials in 1997. Amer. J. Potato Res. V. 79 p. 451-457.

Simko, I., Costanzo, S., Haynes, K., Christ, B., Jones, R.W. 2004. Linkage disequilibrium mapping of a *Verticillium dahliae* resistance quantitative trait locus in

tetraploid potato *(Solanum tuberosum)* through a candidate gene approach. Theor. Appl. Genet. V. 108 p. 217-224.

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Simko, I., Haynes, K.G., Ewing, E.E., Costanzo, S., Christ, B..J., Jones, R.W. 2004. Mapping genes for resistance to *Verticillium albo-atrum* in tetraploid and diploid potato populations using haplotype association tests and genetic linkage analysis. Mol. Gen. Genomics. V. 271 p. 522-531.

Cereal Rusts Resistance II

Background: The cereal rust fungi *Puccinia graminis* (stem rust of wheat, barley, and oat), *P. triticina* (leaf rust of wheat) and *P. coronata* (crown rust of oat) are widespread pathogens of cereal crops throughout the United States and world-wide. The rust fungi are highly variable, as many different physiologic races exist with the ability to cause disease on host cultivars with different combinations of resistance genes. Stable rust resistance in cereal crops has been difficult to achieve due to the high variation for virulence present in rust populations. Genetic characterization of cereal rust fungi, and identification of rust resistance genes in cereals can assist in the development of crop cultivars with durable resistance.

Accomplishment: Leaf rust resistance genes in commonly grown hard red spring and soft red winter wheat cultivars were identified by gene postulation methods in collaboration with the University of Minnesota. The combinations of genes that conditioned the best leaf rust resistance was identified in greenhouse tests with seedling plants and in field plot tests. Genetic analysis determined that wheat cultivars with leaf rust resistance genes *Lr13, Lr16, Lr23,* and *Lr34,* had very good leaf rust resistance. Genetic analysis of selected wheat germplasm lines has indicated the possible presence of new leaf rust resistance genes that condition resistance to a wide range of leaf rust races. Wheat germplasm lines with combinations of adult plant resistance genes that condition high levels non-specific durable leaf rust resistance have been developed.

Impact: The rust fungi that attack small grain cereal crops are highly variable and dynamic pathogens that often overcome disease resistance genes in cereals. Proper identification and characterization of rusts that attack wheat, oats, and barley, will enable plant pathologists and plant breeders to select crop genotypes with combinations of resistance genes that will provide more durable resistance to the rust pathogens. Identification of rust resistance genes that provide durable resistance and molecular markers associated with these genes will assist in the development of crop cultivars with durable resistance. This research will result in reduced crop losses caused by cereal rust diseases.

Documentation:

Argrama, H., L. Dahleen, M. Wentz, Y. Jin, and B. Steffenson. 2004. Molecular mapping of the crown rust resistance gene *Rpc1* in barley. Phytopathology 94:858-861.

Leonard, K.J., Anikster, Y., and Manisterski, J. 2004. Patterns of virulence in natural populations of *Puccinia coronata* on wild oat in Israel and in agricultural populations on cultivated oat in the United States. Phytopathology 94:505-514.

Anikster, Y., Szabo, L. J., Eilam, T., Manisterski, J., Koike, S. T., and Bushnell, W. R. 2004. Morphology, life cycle biology and DNA sequence analysis of rust fungi on garlic and chives from California. Phytopathology 94: 569-577.

Kolmer, J. A., Long, D. L., and Hughes, M. E. 2004. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2002. Plant Dis. 88: 1079-1084.

Oelke, L. M. and Kolmer, J.A. 2004. Characterization of leaf rust resistance in hard red spring wheat cultivars. Plant Dis. 88: 1127-1133.

Bean Virus Resistance

Background: Virus diseases of bean (*Phaseolus vulgaris* L.) caused by bean common mosaic virus (BCMV), bean common mosaic necrosis virus (BCMNV), and Beet Curly Top Virus (BCTV) are responsible for significant global economic losses. The most effective genetic resistance to the potyviruses BCMV and BCMNV results from an interaction between two resistance genes, *bc-1*^{2} and *I*. Two molecular markers, $SBD5_{1300}$ and $SW13_{690}$ have been developed to identify plants that have *bc-1*^{2} and *I*, respectively. However, these two markers are dominant markers and cannot be used to distinguish between heterozygous and homozygous plants. To accurately identify plants with most desired resistance gene combination (*bc-1*^{2} *bc-1*^{2} *I I*) it has been necessary to self-pollinate plants identified with the dominant molecular markers and evaluate progeny plants in the greenhouse for virus resistance, a process requiring at least four months for completion. No molecular markers have been identified that can be used to select for resistance to BCTV. Disease screening currently relies on field tests that are dependent on the presence of virulent leafhoppers, the vector of BCTV, and effective screening can require several years of field tests. The need to conduct greenhouse or field evaluations for virus resistance results in considerable delays and expenses to bean breeding programs. A new method will be developed for accurately detecting as seedlings, plants with the *bc-1*^{2} *bc-1*^{2} *I I* genotype. A molecular marker will be identified that can be used to rapidly screen beans for resistance to BCTV.

Accomplishments: A multiplex real-time PCR assay was developed to simultaneously genotype bean plants for the resistance genes *I* and *bc-1*^{2}. Real-time PCR assays were developed that were specific for each gene, multiplex PCR reaction conditions were optimized, and a method of data analysis was developed to genotype seedlings based on

PCR results. The assay could unambiguously assign F_2 plants to one of nine genotypes. The accuracy of the assay was validated by also genotyping all dF_2 plants for the *I* and *bc-1*^{2} genes by performing F_3 family progeny tests for virus resistance. The multiplex real-time PCR assay was 100% accurate in genotyping plants for the *bc-1*^{2} gene and was 92.4% accurate (183/198) for the *I* gene.

A sequence characterized amplified region (SCAR) marker, *AS8*$_{1550}$, was identified that is tightly linked to the *Bct* gene, which conditions resistance in bean to BCTV. This marker can accurately screen snap and dry bean seedlings from the Andean *Phaseolus* gene pool for the *Bct* gene.

Impact: Greenhouse and field evaluations for virus resistance in beans are costly and can require from months to years for completion. Successful completion of these tests can also be limited by the presence of environmental factors that inhibit processes of plant infection and symptom expression. The *AS8*$_{1550}$ marker provides bean breeders with an accurate and rapid method for identifying Cranberry, Kidney and Snap beans that have the *Bct* gene for resistance to BCTV. This will provide an alternative screening procedure to field evaluations, which can be highly compromised by the absence of the beet leaf hopper (*Circulifer tenellus*) of BCTV.

The multiplex real-time PCR assay developed to simultaneously genotype bean plants for the resistance genes *I* and *bc-1*^{2} will drastically reduce requirements for progeny testing plants for resistance to BCMV and BCMNV. This assay can be used in conjunction with the *AS8*$_{1550}$ marker to rapidly identify plants having genes conferring resistance to MBMV, BCMNV, and BCTV. Molecular markers linked to genes responsible for desired agronomic traits are very useful tools for accelerating population improvement in many important crop species. Unfortunately, the great majority of useful molecular markers are dominant. In the course of developing the multiplex real-time PCR assay for *I* and *bc-1*^{2}, ARS has identified a general experimental protocol that can be applied to developing real-time PCR assays for the codominant interpretation of dominant markers in other diploid plants.

Documentation:

Larsen, R., Miklas, P. 2004. Generation and Molecular Mapping of a SCAR Linked with the *Bct* Gene for Resistance to Beet Curly Top Virus in Common Bean. Phytopathology. V. 94:320-325.

Vandemark, G., Barker, B. 2003. Quantifying *Phytophthora medicaginis* in susceptible and resistant alfalfa with a real-time fluorescent PCR assay. Journal of Phytopathology. V. 151:577-583.

Vandemark, G., Miklas, P. 2002. A fluorescent PCR assay for the codominant interpretation of a dominant SCAR marker linked to the virus resistance gene *bc-1*^{2} in common bean. Molecular Breeding. V.10: 193-201.

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Rice Magnaporthe Resistance

Background: Fungal pathogens of plants are the most devastating plant disease agents. ARS is studying the molecular biology of host recognition of the model plant pathogenic fungus, *Magnaporthe grisea*, by rice. This fungus is considered a premier example of an ascomycete fungal plant pathogen, which can be manipulated both genetically and with the tools of molecular biology. Likewise, rice is considered to be the model monocot plant with a small, diploid genome that can be readily modified by transformation. The genomes of both organisms have been sequenced.

Rice Blast Disease caused by *M. grisea* is the most important disease of rice worldwide causing 11-30% loss in yield and reducing grain quality in many rice-growing regions of the world. In the United States rice blast is a sporadic but important problem when it occurs. For example, leaf blast disease has been found in an estimated 400,000 of 1.5 million acres of rice planted in Arkansas this year.

Rice is the staple crop of two thirds of the human population and major increases in population are expected in rice-consuming countries over the next century. Thus rice blast disease is a significant constraint to global food security and agricultural trade.

M. grisea causes also attacks other important cereals and grasses such as wheat causing wheat blast and perennial rye causing grey leaf spot. Similar genes are often required for infection of different host plants by *Magnaporthe grisea.* Thus, information can be anticipated that is broadly applicable to fungal pathogenesis of grasses will be obtained through studies of this model fungus and its interaction with rice. The evolutionary relationship of fungal host and cultivar specificity genes and their corresponding disease resistance loci is currently unknown and may be exploited to develop new disease resistance specificities using transgenic and wide hybridization technologies. The use of the cloned *AVR1-CO39* gene as an adjuvant to promote plant health remains largely unexplored.

Accomplishment: The most effective method for control of rice blast is to grow disease resistant plants. Unfortunately, *M. grisea* is able to overcome this resistance within 1-3 years after resistant plants are cultivated widely. ARS is trying to understand the molecular details of how the rice blast fungus is recognized by rice plants that are resistant to blast and how the fungus changes in order to overcome this recognition. In order to improve our understanding of this host-parasite interaction, ARS scientists have cloned a pathogen gene AVR1-CO39 that is involved in recognition of the pathogen by

the resistant rice plant. This is the second AVR (AViRulence) gene to be cloned from the rice blast fungus. A combination of physical, genetic and molecular genetic methods were employed to obtain the cloned gene. These methods included our laboratory's high density genetic map and molecular karyotype, targeted genome cleavage methods, and a transformation system developed for *M. grisea* that is based on drug resistance. Considerable insight was gained about the map-based cloning approaches through this effort. These approaches and insights will likely have broad application to other organisms such as the cereal rusts in which map-based cloning approaches are being used to clone similar genes. In parallel work, ARS has mapped and cloned the corresponding disease resistance locus Pi-CO39 (t), which allows the host to recognize strains of the fungus that carry AVR1-CO39. This work has shown that resistance is controlled by a dominant locus mapping to chromosome 11 of rice. The complete DNA sequence of the locus from two rice genotypes has been completed, annotated, and analyzed for transcriptional activity. Annotation of 416 kb of DNA sequence from rice variety Nipponbare and 231 kb from variety CO39 linked to Pi-CO39(t) locus, that contains the putative receptor for the M. grisea avirulence gene AVR1-CO39, revealed the presence of several NBS-LRR, receptor kinase, and Serpin genes in both haplotypes. The long term goal is to study the molecular biology of the interaction of Avr1-CO39p and the host resistance gene product and/or other host products in order to understand when, where and how the fungus communicates its presence to the host. Tissue culture methods for induction of callus from rice leaf explants and production of plantlets from this callus have been developed as a first step to transform the plastid. Using this technology, transfer and containment of host resistance and pathogen avirulence genes to rice can be achieved.

Impact: This research has led to the identification of the polypeptide component of AVR1-CO39 that elicits a PiCO39 (t)-dependent response and the tentative identification of two genes at the PiCO39 (t) locus that function in the disease resistance response in rice. Based on this information, it is hoped that new strategies for engineering novel kinds of broad-spectrum resistance to M. grisea in rice and other cereals and grasses of economic and ornamental value will be developed. A novel tissue culture method for the isolation of callus from leaf explants of rice has been developed as a first step toward transformation of rice plastids. The AVR gene has been licensed to Kansas State University for collaborative studies to deliver the gene product to rice from rice-associated bacteria.

Additional Information: Extramural funding to identify DNA polymorphisms for genetic analysis of QTLs controlling milling quality and sheath blight resistance in U.S. rice varieties is newly supported by the NRI program for applied genomics.

Documentation:

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Potato Genome Project

Background: The Potato Genome Project http://www.potatogenome.org/nsf4/index.php, funded by the National Science Foundation, conducts fundamental research and provides a comprehensive set of structural and functional genomic resources to further the understanding of potato biology. The project also fosters public awareness and science education in plant genomics. The research goal of the Potato Genome Project is to contribute to the understanding of important features of potato biology including disease resistance, responses to abiotic stress, tuber development, heterozygosity, and polyploidy. A major research focus of the Potato Genome Project is dissection of the structure, function and evolution of pathogen resistance genes (R genes) and "resistance hot spots", conserved chromosomal locations in Solanaceae where several R genes, R gene homologs and genetically defined pathogen resistance loci are clustered. Understanding the structure of pathogen resistance loci will provide insight into the structure-function relationships of R genes, the capacity of individual R proteins to recognize specific strains of pathogens.

Accomplishments: Physical maps and generated annotated genomic sequence http://www.tigr.org/tigr-scripts/tdb/potato/BAC_annotation/bac_display.pl of pathogen resistance hotspots of the hexaploid wild potato species, *S. demissum*, the origin of several *P. infestans* resistance traits used in cultivated potato have been constructed. Maps were constructed for each of the three S. demissum genomes (haplotypes, homeologues) on the lower arm of chromosome 11 and the upper arm of chromosome 5. The three chromosome 11 maps each span ~2.5 Mb and include over 30 R gene sequences per genome. At least three active *P. infestans* resistance genes, R3a, R3b, and R7 have been mapped to this chromosomal region and 0.5 Mb of genomic DNA sequence of one genome is available (see above link). Comparisons between the physical maps and available sequence of the chromosome 11 R gene hotspots of *S. demissum and S. tuberosum* (wild and cultivated potato) reveal remarkable conservation and suggest mechanisms for generation of multiple disease resistance traits in this region.

The chromosome 5 S. demissum maps each span about 2 Mb, with varying numbers (3-30) of R gene sequences per genome. The *R1 P. infestans* resistance gene, the *Nb* virus (PVX) resistance gene and QTLs for *P. infestans* and nematode resistance have been located in this region. Approximately 2 Mb of sequence from the three homeologous genomes (haplotypes) is available from this region. Structural comparisons in this region reveal three classes of R genes and frequent sequence exchanges between the *R1* class homologues.

The important *P. infestans* resistance locus, RB and three RB pseudogenes, from the wild species *S. bulbocastanum*, on chromosome 8, were also isolated and sequenced. Test for RB conferring late blight resistance in cultivated species are underway.

In addition to conducting research on important aspects of potato biology the Potato Genome Project provides the scientific and agricultural communities with a set of resources that aid other research efforts and the development of new agricultural practices http://www.potatogenome.org/nsf4/tigr_resources/, http://www.tigr.org/tdb/potato/. These comprehensive resources include: a database of annotated genomic sequence of potato and other Solanaceae pathogen resistance hot spots, the SOLAR database of R gene sequences and an SSR database. Functional genomic resources include publicly available potato *and N. benthamiana* EST sequence and gene indexes, 10K potato cDNA microarrays, a Solanaceae expression profiling service and the Solanaceae gene expression database providing the first comprehensive resource for gene expression patterns in the Solanaceae family.

A functional genomics resource of genes involved in pathogen resistance in Solanaceae is being constructed. The database will provide results from gene silencing experiments of genes that play a role in disease resistance using virus induced gene silencing and stable silencing. Project participants have recently described successful deployment of virus induced gene VIGS silencing in wild potato. This result opens the way effective method of rapidly assessing gene function in wild and cultivated Solanum and Solanaceae.

The Center for Plant Genomics Training and Education http://outreach.potatogenome.org/ hosts summer genomics workshops designed to provide underrepresented high school and college students with the opportunity to conduct a unique question-based genomics experiment with real world applicability. The center has helped establish community gardens that serve to educate students, teachers, researchers and the public about plant genomics, Solanaceous crops, the importance of plant diversity and applications of genomics for breeding sustainable, environmentally friendly crops.

Impact: As the fourth most important contributor to human calorie consumption, potato bears an enormous influence worldwide. The global influence and impact of potato make it important and relevant to plant biology and genome research.

Additional Information: Funding was received from NSF Plant Genome Research Program from 1999-2002 (DBI99866) and from 2002-2007 (DBI027856) for a

collaborative research project with ARS and non-ARS scientists. Collaborators are TIGR; ARS, Prosser, Washington; Cornell University; University of Wisconsin, Madison; University of Minnesota; Sainsbury Laboratory, UK; and Universidad Nacional de Rosario, Ar.

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Discovery Area 3: Mechanisms of Resistance

Background: Knowledge of resistance mechanisms is essential to develop novel and rational approaches to disease management. Although our understanding of resistance genes has advanced substantially during the past few years, the identities and functions of the proteins that interact to confer resistance are just beginning to be understood. The recent molecular cloning of several plant genes that confer disease resistance to a diverse range of pathogens has revealed that the encoded proteins have several features in common, suggesting that plants may have evolved common signal transduction mechanisms for expressing disease resistance to a wide range of unrelated pathogens.

The characterization of the molecular signals involved in pathogen recognition and the elucidation of the molecular events specifying the expression of resistance may lead to the development of novel strategies for disease control.

Gene Silencing in Wheat

Background: Wheat is one of the most important crops in the world's food supply. Unfortunately, the identification of and the functional analysis of genes, which are essential steps in genetic improvement of wheat through the application of the tools of molecular biology, are difficult and slow for several reasons. The gene redundancy due to wheat having a hexaploid genome, its extremely large genome size and being very difficult to transform essentially eliminates many of the gene isolation tools such as such as T-DNA mutant libraries in diploid lines, transposon mutation systems and generating gene knockout by stably integrated RNA-interference constructs. ARS developed a tool that is very effective in circumventing these obstacles. The tool is a virus-induced gene silencing system, which will permit the rapid creation of gene knockouts that can be used to establish gene identities and functions. This process relies of infecting wheat with a virus, rather than the creation of transgenic plants. Infection of wheat with an RNA virus that has been engineered to carry a small piece of a plant gene causes activation of the plants sequence-dependent RNA degradation system and results in targeting all of the viral RNA for destruction. However, because there is a plant sequence within the viral genome, any plant RNAs with related sequences are also degraded. This system is very well suited for use in wheat because it will silence any other copies of a gene present in the hexaploid genome as long as they are very similar in nucleotide sequence.

Accomplishment: ARS has developed a wheat VIGS system using vectors based on barley stripe mosaic virus (BSMV). This system has been employed to functionally identify genes required for resistance to leaf rust mediated by the Lr21 R-gene. It was shown that infecting resistant lines with BSMV carrying a portion of Lr21 caused conversion to susceptibility, while infection with BSMV with no plant gene had no effect. Additionally, three other genes that are known to be required in some, but not all resistance pathways of Arabidopsis, tomato, tobacco and barley might also function in Lr21-mediate resistance. Infection of resistance wheat with BSMV carrying portions of RAR1, SGT1 and HSP90 all caused resistant lines to become susceptible.

Impact: Development of a convenient tool for gene silencing in wheat is highly significant for anyone performing genetic analysis in wheat, whether in an academic or industrial setting. It provides a new and rapid strategy for gene identification and functional analysis that overcomes many of the obstacles that currently confound wheat research. In particular, this tool will be very useful to identify genes encoding products that are essential in disease resistance. Knowledge of such genes is essential in efforts to engineer improved disease resistance in wheat.

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Soybean Microarrays

Background: Major diseases of soybean are caused by diseases for which no single gene resistance is known and defense depends on activities of multiple genes. Identification of genes involved in defense against pathogens is difficult when quantitative trait loci (QTL) are involved and each genetic locus provides only a fraction of the defense. The use of microarrays can allow one to identify defense-related genes by studying the differentially expression between resistant and susceptible plants. To develop a better understanding of defense against pathogens in soybean, and to increase chances of identifying defense QTLs, ARS have used microarrays to characterize the genomic response to several virulent and avirulent pathogens as well as a symbiont.

Accomplishment: ARS has characterized the genomic response in soybean to virulent and avirulent strains of *Pseudomonas syringae* pv *glycinea*. The research lends further support to the developing theory that plants tap into the power of chloroplast to generate reactive oxygen species to defend against pathogens and that the timing of this response is critical to defense. This project, in addition to ones on the response of soybean to aphid, *Fusarium*, *Sclerotinia*, and *Rhizobium* are allowing us to identify genes involved in soybean's response to pathogen and symbionts and provide hopes of identifying genes of QTLs.

Impact: Genomic studies from these and future projects promise to lead to a deeper understanding to biology of resistance and will allow research to focus in on key triggers

of defense. By combining gene expression studies with mapping, one has to potential of linking a differentially expressed gene with association to a QTL map location and possibly to identity of the underlying gene.

Additional Information: Collaboration with the University of Illinois contributed to the research leading to accepted publications during this review period. Research being undertaken but which has yet to lead to publications during this period, is being assisted by funding from USDA-CREES (The National Sclerotinia Initiative and the University of Illinois Soybean Disease Biotechnology Center), DOE (effects of global change on disease), United Soybean Board (Soybean Rust), and via collaborations with Ag-Canada (Sclerotinia infections and RNA isolations), University of Illinois (photosynthesis imaging of diseased tissue), ARS Urbana (pathogen inoculations and sharing of postdoc and graduate student), and University of Illinois (provider of cDNA microarray slides).

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Virulence Factors for Corn Gray Leaf Spot

Background: Gray leaf spot (GLS) of corn, caused by *Cercospora zeae-maydis*, has increased in frequency, distribution, and severity during the past two decades and is the major foliar disease of corn, causing substantial economic losses in the United States, Africa, and South America. The increased incidence and severity of GLS are associated

with conservation tillage practices and monoculture of corn, which result in a build up and survival of inoculum in debris for early infection of the subsequent crop. Most commercial hybrids are moderately to highly susceptible to *C. zeae-maydis* and vary widely in their disease reaction from location to location, either as a result of genetic diversity in the pathogen population or major environmental effects. Published estimates of yield reduction due to GLS range from 2% to >40%. Thus, a conservative estimate of 5% yield reduction translates into a loss of over 250 million bushels and an economic impact of several hundred million dollars in the four major corn-producing states in the midwestern United States alone (Iowa, Illinois, Indiana, Nebraska), where nearly 50% of the Nation's corn is produced. In seed production fields, additional costs are incurred by the application of fungicides used to reduce the damage from *C. zeae-maydis*. Like many species of *Cercospora*, *C. zeae-maydis* produces cercosporin, a low molecular weight, lipid-soluble perylenequinone that localizes in host cell membranes and, thus, is toxic to a diversity of organisms beyond the host range of the producing fungal species. Cercosporin affects plant cells through the light-activated generation of active oxygen species, predominantly singlet oxygen, which results in lipid peroxidation, membrane damage, leakage of nutrients, physical stress, and eventually cell death. The role of cercosporin in GLS had not been determined.

Accomplishment: Research on the previous CRIS project established that GLS of corn is caused by two sibling species of *Cercospora zeae-maydis*. Two genetically distinct but morphologically indistinguishable forms of the pathogen were isolated from diseased leaves of corn in a national GLS survey. Members of one group of the pathogen were found throughout the corn-producing regions of the United States, whereas members of the other group were localized in the eastern third of the country. During the current research period, global populations of the GLS pathogen were analyzed. Results of analyses comparing DNA profiles of the pathogen from Africa and South America indicated that both forms of the pathogen are present in South America, but the pathogen population in Africa is comprised exclusively of the less prevalent form that is localized in the eastern part of the United States. Because members of the two groups are morphologically indistinct yet cause identical disease symptoms, a rapid molecular (PCR-based) method was developed to distinguish each sibling species as well as other fungal foliar pathogens of corn and sorghum. The method, which detects differences in the nucleotide sequences within the internal transcribed spacer (ITS) regions of ribosomal DNA, reduces the time and steps that are required to identify the pathogen and provides definitive criteria that are applicable without relying on variable and overlapping taxonomic criteria obtained from cultures *in vitro*.

Toward assessing the importance and essentiality of cercosporin production by *C. zeae-maydis*, we determined nutritional conditions that induce or suppress cercosporin synthesis in culture and developed methods for genetic transformation and gene disruption of the pathogen. By cDNA subtraction hybridization methods, ARS identified and analyzed genes that are uniquely expressed during cercosporin biosynthesis. Disruption of genes that were predicted to be critical for cercosporin synthesis established that cercosporin is a virulence factor that is essential for the fungus to cause GLS symptoms.

Impact: This information impacts the screening of corn germplasm for GLS resistance and the development of resistant hybrids via traditional breeding or genetic engineering strategies designed to control GLS by conferring resistance to cercosporin. Analysis of the pathogen population will provide clues to the mechanism of change in this fungus and its relatives that are pathogens of other grain crops. Based on information on the global distribution of the pathogen, a tenable hypothesis was proposed to explain the source of the second sibling species in the United States and its phylogenetic relationships with other *Cercospora* and *Mycosphaerella* species. The simple and reliable PCR method of distinguishing between the sibling species was shared with other investigators in South America and Africa, who applied the method to identify isolates of the GLS pathogen and determine genetic diversity in resident populations on those continents. The results of this work were featured in several popular press articles. Numerous investigators from the United Kingdom, Africa, Denmark, and South America as well as Mexico and the United States requested specific information about the pathogen and its potential impact on disease severity; others requested cultures of the pathogens.

Additional Information: Several collaborators assisted with collections of GLS samples of corn from Brazil, Peru, Colombia, Uganda, Zimbabwe, Kenya, and South Africa. A colleague at Purdue University provided expertise in the population genetic analysis of the GLS pathogen.

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Resistance Factors in Sweetpotato

Background: Sweetpotato varieties developed by are resistant to several insects and diseases; however, the mechanisms of pest resistance in sweetpotato are not understood. Screening large numbers of individual clones for resistance is laborious and imprecise. Understanding the role of sweetpotato constituent compounds in resistance may prove useful, because they could be used as markers to rapidly identify resistant clones.

Accomplishments: Studies were conducted to characterize and quantify secondary constituents in sweetpotato storage roots and assess their biological activity in bioassays. Storage root contents of several secondary compounds were determined for a genetically diverse sweetpotato germplasm collection. Sweetpotatoes varied in contents of resin glycosides, caffeic acid, chlorogenic acid, three isomers of dicaffeoyl quinic acid, scopolin, scopoletin, p-coumaric acid and three isomers of galactosyl diglyceride. Bioassays were used to test the activity of these constituents against four root rotting fungi (*Fusarium oxysporum, F. solani, Lasiodiplodia theobromae,* and *Rhizopus stolonifer*) and the bacterial root rot pathogen, *Erwinia chrysanthemi*. Caffeic acid inhibited the fungi and bacteria at levels found in the periderm of some sweetpotato clones. Chlorogenic acid was inhibitory to the fungi at constituent levels, but it was not highly inhibitory to the bacterium. Periderm resin glycosides were inhibitory to fungi. Scopoletin inhibited the fungi, but its glycoside, scopolin was inactive. Fungal species differed in sensitivity to the phenolic compounds and the periderm resin glycosides. A new class of compounds discovered in sweetpotato, galactosyl diglycerides, inhibited insect growth and seed germination but was not active in fungal or bacterial bioassays.

Impact: Identification of resistance factors in sweetpotato may lead to chemical assays useful in identifying resistant germplasm. Sweetpotato researchers in Alabama and Louisiana have initiated projects that are attempting to use resin glycosides as a marker for resistance. Reducing sweetpotato losses due to rot diseases in the field or storage is the long term objective. Elucidating biological activities for sweetpotato constituents may lead to the discovery of useful biological activities for natural products.

Additional Information: Cooperation with the Plant Pathologist at Louisiana State University was instrumental in developing bioassay protocols and providing pathogen cultures.

Documentation:

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Nematode Resistance Factors in Soybean

Background: Soybean is a major crop in the United States and the soybean cyst nematode is the most important pest of this crop. The soybean cyst nematode is responsible for an estimated one billion dollars in losses for the U.S. farmer annually and affects most soybean growers in the United States. To maintain a competitive edge in the world soybean market, the U.S. farmer needs soybean with broad resistance to many different races of soybean cyst nematode. Soybean genes important to resistance against the soybean cyst nematode are being identified and characterized to determine their role in resistance. New and innovative approaches are being taken to develop new strategies for broadening the resistance of soybean to the different races of soybean cyst nematode.

Accomplishment: Little is known about the resistance and susceptible responses of soybean to the soybean cyst nematode. Differences between the resistance response and susceptible response may provide clues as to how the soybean plant defends itself against nematodes. In turn, this information may be useful in developing new strategies to provide soybean with broad resistance to the numerous races of soybean cyst nematode found in the United States. Gene expression in soybean roots susceptible to the soybean cyst nematode was examined over the first eight days of nematode infection to identify genes that may be useful in broadening resistance to the soybean cyst nematode. RNA was harvested from soybean roots susceptible to infection by the soybean cyst nematode at different time intervals after nematode invasion from 6 hours to 8 days and applied to a targeted microarray consisting of approximately 6,000 cDNAs, derived from soybean root libraries or selected from other libraries, because they may be involved in the defense response. Numerous genes were identified that may be important to the interactions of soybean root with the soybean cyst nematode.

Impact: This data will aid scientists in identifying soybean genes involved in susceptibility and resistance to the soybean cyst nematode, so soybean can be engineered with broader resistance to the soybean cyst nematode in the future.

Additional Information: United Soybean Board provided financial support and Dupont provided over 1,000 clones to add to the microarray. This provides a broader range of

soybean genes to survey and provided a postdoctoral research associate so the work could be accomplished much more quickly.

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Gene Silencing in Beans

Background: The quality of crops by identifying genes involved in agronomically important traits such as disease resistance and incorporating these genes into breeding programs were identified. Virus-induced gene silencing (VIGS) is a tool with tremendous potential for identifying these genes.

Accomplishments: The model organism of study is *P. syringae* pv. syringae, the causal agent of brown spot disease of bean. It has been established that the *gacS* and *gacA* genes are required for disease on bean. ARS has characterized numerous genes within the *gacS/gacA* regulon. Sequencing of the 28 kb tabtoxin biosynthetic region, this toxin is highly damaging to plants and is regulated by *gacS/gacA*. Methodologies have been developed to uniquely mark bacteria with antibiotic resistances for study in the field. ARS has successfully applied VIGS as a tool for genetic analysis in tomato and has developed methodologies for the quantitation of gene expression in plants using Real-Time qrt-PCR.

Impact: Analysis of the *gacS/gacA* regulon impacts research focused on understanding the genetics of disease caused by both plant and animal pathogens. Development of a unique method to “mark” bacterial strains with easily distinguishable antibiotic

resistances allows the study of the epidemiology of bacterial diseases in the field environment and allows for the improvement in sampling techniques to detect disease. The sequence of the tabtoxin biosynthetic cluster is of major interest to scientists studying antibiotic resistance mechanisms. The development of Real-Time qrt-PCR techniques to analyze VIGS in plants allows us to use this powerful method to study gene action in tomato and potato. The ultimate goal is to improve disease resistance in agronomically significant crops through the identification of important genes. These identified genes can then be used in breeding programs to produce disease resistant cultivars.

Additional Information: Work with VIGS in tomato was accomplished through a Specific Cooperative Agreement with the Department of Entomology at the University of Wisconsin-Madison.

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Barley GeneChips

Background: Fungal pathogens are perhaps the greatest impediment to production of grain cereal plants worldwide. When environmental conditions are opportune for pathogen growth, between 30 and 50% of potential yield can be lost. To offset crop losses from pathogens, plant breeders have turned to genetic varieties that help crops resist these diseases. Barley powdery mildew was used as the initial model system. The disease is caused by the obligate fungal pathogen, *Blumeria graminis,* and is widely distributed throughout the world. It is most damaging in cool, wet climates, such as

Northern Europe and the winter growing season in the Mid-Atlantic and Southeastern United States. Powdery mildew disease causes reduced grain yield, kernel weight and grain protein. Excellent genetic materials and availability of cloned genes specifying resistance to this disease make this system ideal for basic studies relating to recognition and active defense.

Accomplishments: ARS was co-leader in the development of Barley1 GeneChip: A worldwide uniform platform to investigate the function of 22,000 cereal genes in a single experiment. Barley1 allows investigators to use cutting-edge genomics technology to improve disease defense, responses to abiotic stresses, yield, and biodiversity. Already, Barley1 has been used to discover new genes for resistance to Fusarium head blight, powdery mildew, and stem rust, diseases that are among the greatest deterrents to small grain production. Barley1 is also serving as a blueprint for new wheat, soybean, grape, rice, and corn GeneChips.

Impact: 2,000 Barley1 GeneChips have been distributed since June 2003; 1/3 to International investigators. In addition, ~20 jobs have been created as a direct result of this project; at least 20 more from spinoffs. The masses of data (65 Mb/chip) resulting from use of the publicly available Barley1 GeneChip are hosted in the NRI-funded BarleyBase database (http://www.barleybase.org/), facilitating new gene discovery and utilization. Since its full inception in October, 2003, BarleyBase has been accessed from over 60 countries in Europe, North America, and the Pacific Rim; 70 registered users in 15 countries have batch downloaded data from complete experiments 360 times for analysis. BarleyBase currently hosts 30 completed experiment submissions with a total of 1,079 hybridizations from seven different Affymetrix Plant Genome Arrays. BarleyBase has become a prototype for gene expression databases and the system architecture will be used for the development of PLEXdb, a unified public resource for large-scale plant gene expression data. This integrated effort will facilitate hypothesis building by the analysis of high-throughput parallel expression experiments.

The Corn Insects and Crop Genetics Research Unit, five U.S. universities (Iowa State University, University of California-Riverside, University of Arizona, University of Minnesota, Washington State University), and institutions from five countries on four continents partnered in this project. Supported in part by USDA - Initiative for Future Agricultural Systems grant no. 2001-52100-11346.

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