

USDA  
AGRICULTURAL RESEARCH SERVICE

**NATIONAL PROGRAM 303 – PLANT DISEASES**

**ANNUAL REPORT FY 2008**



# National Program 303 – PLANT DISEASES

## FY 2008 Annual Report

### *Introduction*

National Program 303, Plant Diseases, focuses on developing effective disease control strategies that are environmentally friendly, do not threaten the safety of consumers, and are compatible with sustainable and profitable crop production. This ARS National Program is conducted in cooperation with related research in other public and private institutions. In addition, NP 303 projects are coordinated with those in NP 301 (Plant Genetic Resource, Genomics, and Genetic Improvement) and NP 302 (Plant Biological and Molecular Processes) toward the overall goal of crop improvement through increased resistance to biotic and abiotic factors and increased understanding of host-pathogen interactions.

The overall goal of NP 303 is to develop and improve ways to reduce crop losses caused by plant diseases. These diseases reduce yields, lower product quality or shelf-life, decrease aesthetic or nutritional value, and, sometimes, contaminate food and feed with toxic compounds. Control of plant diseases is essential for providing an adequate supply of food, feed, fiber, and aesthetics. Reducing these losses has long been a high priority for agriculture and for the Agricultural Research Service (ARS). Besides the obvious monetary benefits to producers and processors, successful plant health protection is important for maintaining and increasing food supplies with minimal increases in land under cultivation. Additionally, the 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.

The Action Plan for NP 303 was written with input from a customer and stakeholder planning workshop in October 2005 to ensure that the components of the program were relevant to current needs of U.S. agriculture. Teams of ARS scientists worked with National Program Leaders to develop this Action Plan that provides a framework for ARS research on plant diseases over a 5-year period. Four component areas were identified by customers and ARS scientists at the workshop. Each of the four components contains problem areas that require research. Based on this Action Plan, ARS scientists wrote project plans describing research they will conduct; the anticipated products or information to be generated by the research; the roles and responsibilities of ARS scientists and their cooperators; and the timelines and milestones to measure progress toward achieving the goals of the research.

NP 303 is comprised of the following four components:

- *Disease Diagnosis: Detection, Identification and Characterization of Plant Pathogens;*
- *Biology, Ecology, Epidemiology, and Spread of Plant Pathogens and their Relationships with Hosts and Vectors;*
- *Plant Disease Resistance;* and

- *Biological and Cultural Strategies for Sustainable Disease Management.*

Together, these components are yielding breakthroughs in understanding and controlling plant diseases and in developing strategies for controlling disease that enhance agricultural value. During fiscal year 2008 this program produced many important discoveries and advances. Some of these are described below, grouped by program component, but not necessarily in order of significance:

### **Component I – Disease Diagnosis: Detection, Identification and Characterization of Plant Pathogens**

- *Detection and differentiation of plant-infecting phytoplasmas.* Over the last few years, numerous emerging and economically limiting plant diseases worldwide have been reported to be associated with diverse phytoplasmas. To aid diagnosis, a computer-simulated genetic analysis system has been developed for identifying and classifying phytoplasmas. Because phytoplasmas are considered to be “fastidious”, and cannot grow outside living hosts, they cannot be diagnosed by traditional methods that are applied to culturable organisms. Over the past decade, establishment of a phytoplasma classification scheme based on DNA analysis made possible the accurate and reliable identification and classification of a wide range of phytoplasmas. ARS researchers in Beltsville, Maryland, expanded this classification scheme through the use of computer-simulated analysis, achieving rapid differentiation and classification of phytoplasmas. This study classified more than 250 previously unclassified phytoplasmas, delineated 10 new phytoplasma groups. This provides a new practical tool for rapid identification of phytoplasma strains and for discovery of novel phytoplasma species. The publication reporting this study was selected by the International Journal of Systematic and Evolutionary Microbiology as a “hot off the press” article and was highlighted by Microbiology Today. This progress will advance work by epidemiologists and other scientists interested in identification of biologically or ecologically distinct phytoplasma strains involved in various important plant diseases.
- *Identification and characterization of new phytoplasma hosts.* Potato purple top (PPT)-related diseases have caused tremendous damage to potato tuber production in South America, Mexico, the United States, and elsewhere in the world. Infected tubers used for potato chips develop brown streaks and discoloration, making them unmarketable. A new phytoplasma strain associated with PPT diseases in Montana and Alaska was identified using new methods developed by ARS researchers in Beltsville, Maryland. In 2007, sporadic diseased potato plants exhibiting symptoms that included rosette and stunting of plants with purple discoloration of top leaves and shoot proliferation were found in a field in Montana and other fields in western Alaska close to the Canadian border. Phytoplasma strains associated with PPT were identified as belonging to members of the milkweed yellows and related phytoplasma strains. This phytoplasma can be transmitted through tubers. This is the first report that this group of phytoplasmas is associated with potato diseases in the United States. This accomplishment will aid implementation of quarantine regulations and

will help extension workers and plant diagnosticians to identify and combat these newly emerging diseases. This progress will be of interest to scientists involved in production of disease-free seed potatoes. In addition to this discovery in potato, new phytoplasmas have been found by these researchers in jujube witches'-broom from northern China, which severely reduces the quantity and quality of jujube production. Hemp fiber is a new host of phytoplasmas in the elm yellows group and a potential reservoir for jujube witches'-broom disease. The findings point to genetic diversity and ecological complexity of phytoplasmas causing jujube witches'-broom disease, and provide new insights for management of jujube witches'-broom disease.

- *Accurate monitoring and prediction of wheat stripe rust in 2008.* Accurate disease forecast is essential for timely management of the economically limiting disease of wheat. In 2008, ARS scientists in Pullman, Washington, conducted long-, mid-, and short-term forecasts for stripe rust epidemics using weather data, disease monitoring data, and cultivar resistance. Stripe rust alerts were sent to growers as early as February and throughout the growing season. As a result of the accurate forecasting, timely alerts, and advice for choosing resistant cultivars and decision support for use of fungicides, wheat growers implemented appropriate measures for stripe rust control. Major yield losses for the hard red winter wheat growing regions in Washington were prevented and multimillion dollar savings were realized by preventing unnecessary use of fungicides in many other regions. In addition, these scientists identified new races of the stripe rust fungus. Like many other pathogens, the stripe rust fungus is able to evolve into new virulent races that can circumvent genetic resistance in wheat and barley cultivars. Predominant stripe rust races were identified from samples collected throughout the United States in 2007. ARS researchers identified 11 new races; some of which are able to overcome resistance conferred by the resistance gene currently used in many wheat breeding programs. Information was sent to breeding programs with a warning not to use that gene alone. The finding of such new races will guide breeding programs to use durable type of resistance and/or combinations of effective major resistance genes to develop cultivars with durable resistance to stripe rust.
- *Two new species in the fungal genus Alternaria were described.* The fungal species Alternaria is important in many crop disease and new species have yet to be identified and described. Two new Alternaria species were described from sunflower seeds and Australian navel oranges. ARS scientists in Wenatchee, Washington, found that these newly published species descriptions contribute incrementally to the list of known species and in the case of navel oranges, describe a new disease of imported navel oranges. This information will be useful for plant quarantine regulatory agencies and to other scientists.
- *Identified Cucumber mosaic virus in cranberry showing 'Funky Flower' symptoms.* Funky flower is a new disease of cranberry that is spreading in Massachusetts and New Jersey. The flowers are misshapen and the plants do not produce fruit. ARS scientists in Corvallis, Oregon, used molecular diagnostics to identify the casual agent. They showed that the plants were infected with Cucumber mosaic virus,

which led to the development of a rapid and sensitive diagnostic assay. This test will be used to monitor the spread of CMV in cranberry and in studies to confirm that CMV is the causal agent. At that point, specific control measures can be implemented to reduce the population of the insects that transmit CMV.

- *Development of a multiplex real time PCR assay for the simultaneous detection of Citrus tristeza virus, citrus stubborn, and Huanglongbing diseases.* California has begun monitoring citrus for the presence of the the three economically limiting diseases: Citrus tristeza virus (CTV), citrus stubborn, and Huanglongbing (HLB), also know as citrus greening. A sensitive molecular assay was developed by ARS scientists in Parlier, California, capable of simultaneously detecting HLB, as well as citrus stubborn and CTV, in any combination from extracts made from citrus tissues. Application of this procedure has potential to develop new information on strains of CTV and distribution of stubborn wherever HLB surveys are taken and can play a role in detection and delimiting surveys for these pathogens.
- *Genetic diversity of Agrobacterium tumefaciens characterized.* Characterization of the genetic diversity of *A. tumefaciens* collected by ARS scientists at Davis, California, across the walnut growing regions has facilitated the development of preplant strategies for the use of biological control agents. In addition, it has dramatically enhanced our forensic ability to identify sources of inoculum which continues to aid the industry as it moves towards the cultivation of *A. tumefaciens*-free planting stock and reduction of post planting infections leading to crown gall formation.
- *A rapid virus detection method for small grains viral pathogens is developed.* Barley and cereal yellow dwarf viruses (B/CYDVs), wheat spindle streak mosaic (WSSMV), soil-borne wheat mosaic virus (SBWMV), and wheat streak mosaic virus (WSMV) constitute the most economically important group of wheat viruses, but methods to identify specific viruses have not been available. ARS researchers at West Lafayette, Indiana, have developed a highly specific and sensitive molecular assay that identifies multiple grain viral pathogens based on their DNA sequence. Using this method the epidemiological basis of these viral diseases is being elucidated through the analysis of samples from Indiana, North Carolina, Arkansas, Georgia, and Missouri. The results have shown that most infections consist of two to four barley and cereal yellow dwarf viruses and have clearly demonstrated that wheat streak mosaic virus, soil-borne wheat mosaic virus, and wheat spindle streak mosaic virus also are important pathogens in the eastern U.S. wheat-growing region. Previously, WSMV was thought to be primarily confined to more arid wheat production areas. This information will be critical in developing wheat, barley, and oat lines that are resistant to an array of viral pathogens. Because of the utility and sensitivity of this method, plant pathogen diagnostic laboratories in six states are adopting this technology as a primary virus diagnostic test. These new methods are providing critical information to plant pathologists and plant breeders that will enable them to develop integrated strategies to reduce the economic impact of these diseases.

- *Genome of an important banana pathogen – *Mycosphaerella fijiensis* – is sequenced.* *Mycosphaerella fijiensis* causes black Sigatoka (or leaf streak) of banana. To aid comparative analyses with a previously sequenced wheat pathogen, the genome sequence of *M. fijiensis* was generated through the Community Sequencing Program of the U.S. Department of Energy's Joint Genome Institute (JGI). The genome sequence contains 73.4 million bases of DNA organized into 395 pieces and coding for approximately 10,327 genes. All available genetic markers were placed on the finished sequence to assess completeness and to identify the correct boundaries between specific proteins. This is the first genome sequence of a banana pathogen and is of great importance to plant pathologists and evolutionary biologists for comparative genomics involving related fungal pathogens in this group, such as the closely related wheat pathogen *M. graminicola*, which was sequenced through the same program previously. These data will ultimately help to improve the resistance of banana crops to limiting diseases.
- *Molecular characterization, detection, and management of *Xylella fastidiosa*, causal agent for Pierce's disease of grape.* Although *Xylella fastidiosa* may be cultured from diseased plants, this is not suited for testing large sample sizes from the field for epidemiological studies. ARS scientists in Parlier, California, developed a rapid, reliable, and high-throughput PCR procedure for *X. fastidiosa*, from diseased almond, a host for another strain of *X. fastidiosa*. The technique will facilitate studies in which infection status for a large number of samples are required to track pathogen spread and disease progression in the field across several species. As with many bacteria, viruses that infect bacteria (phage) also infect *X. fastidiosa*. Virus-like particles resembling phage have been observed infecting stressed cultures of *X. fastidiosa*. These phages hold promise as both a means of disease control and as tools for introduction of foreign DNA to *X. fastidiosa*.
- *Characterization of ribosomal RNA genes from *Candidatus Liberibacter* associated with citrus Huanglongbing disease.* Huanglongbing (HLB) disease of citrus, also known as citrus greening, represents the most serious threat that the U.S. citrus industry has encountered. The disease is associated with a bacterium, *Candidatus Liberibacter* spp, which has not yet been isolated in pure culture. Sequencing of ribosomal RNA genes of the bacterium facilitated development of an improved PCR assay for detection of the bacterium by ARS scientists in Parlier, California. The assay will be useful in studies designed to evaluate the association of the bacterium with HLB disease.

### **Component II – Biology, Ecology, Epidemiology, and Spread of Plant Pathogens and their Relationships with Hosts and Vectors**

- *Functions of proteins of Potato leafroll virus are defined.* Viruses are very simple infectious agents, most of which are composed of DNA or RNA, and a protective coat protein. Potato leafroll virus (PLRV) has two structural proteins that make up its protective coat protein. The minor protein is multifunctional and is known to play a role in spread of the virus both within plant hosts and between plants by insect

vectors. ARS scientists determined how this protein works and have defined what parts of the protein are responsible for different functions. One end of the protein is responsible for restricting the virus to plant vascular tissues, a novel finding that is a first for virology. Furthermore, in this capacity this protein functions as a soluble rather than structure protein. When the protein is incorporated into the shell of the virus, another part of the protein is required for the virus to be transmitted by its insect vectors. Understanding how a virus moves within and between plants provides potential targets for novel control strategies.

- *Specific amino acid positions in the HC-Pro (helper component-protease) protein of wheat streak mosaic virus are critical for mite transmission of the virus.* The helper component protein is required for transmission by aphids of many viruses in the family Potyviridae. Wheat streak mosaic virus (WSMV) is transmitted by mites and belongs to the same group of Potyviruses. Mutations introduced into the HC-Pro coding region of wheat streak mosaic virus were evaluated for systemic infectivity to wheat and vector transmission by the wheat curl mite. Specific amino acid mutations in this protein were identified and shown to be necessary for virus transmission by the wheat curl mite. Mutation at other positions had no effect on vector transmission or resulted in reduced vector transmission efficiency. Results provide direct evidence that vector transmission of WSMV requires a specific site in the HC-Pro. This research provides new information on molecular basis of mite transmission of WSMV, and may ultimately be useful in blocking virus transmission by mites.
- *Ancient isolation and independent evolution of the emerging sudden oak death pathogen *Phytophthora ramorum* is examined.* The exotic pathogen *Phytophthora ramorum* is responsible for extensive mortality of oaks and tanoaks, as well as economic losses to U.S. nurseries due to its infection of common ornamental plants. *P. ramorum* exists as three distinct clonal lineages and it is not clear if they originate from the same, possibly sexually reproducing population of unknown origin. ARS scientists at Corvallis, Oregon, found that the lineages have been diverged for at least 11 percent of their history, an evolutionarily significant amount of time estimated to be on the order of 165,000 to 500,000 years. The divergence of the three clonal lineages of *P. ramorum* supports a scenario in which the three lineages originated from different geographic locations that were sufficiently isolated from each other to allow independent evolution prior to introduction to North America and Europe.
- *Impact of citrus management on California grape growing regions at high risk for Pierce's disease epidemics caused by *Xylella fastidiosa*.* Introduction of the glassy-winged sharpshooter insect (GWSS), the vector of *Xylella fastidiosa*, resulted in destructive epidemics of Pierce's disease (PD) at a magnitude not previously experienced in California. The impact of citrus on PD management is being investigated. Citrus is a favored host of the sharpshooter, and complicates management of PD in grape. ARS scientists in Parlier, California, studied the distribution of grape (PD-susceptible) and citrus (host for sharpshooter) in California and analyzed the historical insecticide application databases using GIS technology. Three counties, Riverside, Kern, and Tulare (where previous outbreaks occurred)

have the highest level of grape-citrus proximities, and appear to be at greatest risk for future epidemics of PD. These findings will facilitate efforts to control Pierce's disease by identifying areas where GWSS vector populations would reach high levels in close proximity to vineyards if the current Area Wide program for GWSS control is abandoned. In the absence of insect control in citrus, GWSS populations reach high levels. They subsequently move into vineyards where they may transmit *X. fastidiosa* to grape. To assess effects of irrigation schedules on feeding preference and reproduction of GWSS on citrus, moderate levels of continuous, reduced irrigation resulted in reduced feeding, lower population levels, and lowered reproduction. Thus, irrigation management may be useful in reducing GWSS populations in citrus when used in conjunction with other cultural/biological control strategies in an integrated pest management program targeting GWSS for PD control.

- *The proteins produced and secreted by plant-parasitic nematodes as they feed play essential roles in nematode infection of host plants.* ARS scientists at Ithaca, New York, have isolated and functionally characterized a chorismate mutase gene that encodes a secreted protein from the golden nematode, an important pest of potato. Importantly, it was discovered that the activity of this secreted protein is controlled by a novel mechanism that has never been reported for any plant-parasitic nematodes. Furthermore, this study found that the chorismate mutase protein is linked to a novel form of nematode resistance in potato. Understanding the function of nematode secretions may lead to the development of novel nematode control strategies.

### **Component III – Plant Disease Resistance**

- *Investigation of a mechanism of highly durable nonhost resistance in grasses.* Southern corn leaf blight is caused by a species of the fungus *Cochliobolus*. Related species create a toxin that is responsible for important diseases of maize and other grain crops. All grass species are resistant to *Cochliobolus carbonum* race 1 (CCR1), and are therefore described as “nonhosts” to CCR1. In some maize lines, CCR1 is a devastating pathogen that can kill plants at all developmental stages. Comparative genomics analysis shows that the resistance genes are a unique development during evolution of the grasses approximately 50 million years ago; all grasses contain these resistance genes, but no related genes are found in other plants. In a collaborative project with Purdue University, ARS scientists developed genetically engineered barley to silence the resistance genes. These experiments clearly demonstrated that these genes were essential for barley's, and almost certainly all other grasses', resistance to CCR1. Without the evolution of Hm genes early in the grass lineage, the emergence of the plants that are now the most significant sources of food and biofuels might have been greatly hindered by CCR1. As well, this genetic resistance is highly durable, functioning over 50 million years, and continues to provide resistance for these essential crops. This work was published in the Proceedings of the National Academy of Science and reported in several pieces in the popular press and radio.



- *Release of sugarbeet germplasms that stand up to attack by the new resistance-breaking strains of Beet necrotic yellow vein virus.* There is a need to develop sugarbeet germplasm with resistance to Beet necrotic yellow vein virus (RB-BNYVV), a new strain of the disease rhizomania of sugar beet. Developed by ARS scientists in Salinas, California, the sugarbeet germplasm lines have resistance to RB-BNYVV, a new strain that overcomes the original source of resistance in beets, which causes rhizomania. Resistance to RB-BNYVV was introgressed from wild beet *Beta vulgaris* subsp. *Maritime*. This resistance was discovered in the germplasm enhancement program at Salinas and confers resistance to RB-BNYVV that defeats widely used Rz1, the original source of resistance. These germplasm resources represent ongoing efforts to combine multiple disease resistance with high productivity and to enhance source populations with genes from wild beet accessions. This germplasm line will give the sugarbeet seed industry, and ultimately the growers and processors, an additional source of resistance to rhizomania.
- *Discovery of powdery mildew resistant bottle gourd.* Powdery mildew is an important foliar disease that can cause severe damage to plants in the pumpkin and watermelon family grown in both open fields and greenhouses. During spring and summer of 2006, we observed that commercial bottle gourd cultivars used as rootstocks for grafted watermelon were severely affected by powdery mildew, reducing their value as a rootstock. ARS scientists in Charleston, South Carolina, tested wild bottle gourd plants collected from different regions of the world for their reactions to powdery mildew in the greenhouse and laboratory. Although none of the plants from the wild bottle gourd collection were completely immune to powdery mildew, several accessions were moderately resistant. These resistant wild bottle gourds should be useful sources of resistance for developing bottle gourd rootstocks with resistance to powdery mildew.
- *Potential soybean rust resistance sources identified and confirmed.* Soybean rust (SBR) causes significant yield losses in soybean in areas where it occurs regularly. In international nurseries, ARS scientists from Urbana, Illinois, evaluated 534 soybean plant introductions (PIs) from maturity groups III through IX that had been selected in greenhouse seedling screens for SBR resistance in a field trial at Centro Regional de Investigación Agrícola in Capitán Miranda, Paraguay, during the 2005-2006 growing season. Two lines were immune in both the field and greenhouse evaluations. In addition, six soybean lines had consistently the lowest level of disease severity across years and locations in Nigeria. In nurseries at the North Florida Research and Education Center (NFREC), 405 PIs were evaluated for resistance to North American SBR isolates in 2007. The resulting data and ratings from a similar trial conducted in Fairhope, Alabama, were similar and confirmed that 103 PIs showed SBR resistance at both locations and at other sites in the Southeast. Adult plant resistance to SBR must be confirmed in multiple locations and years to assess the utility of the soybean lines in breeding efforts. These PIs can be used immediately by breeders (many have already been used in crosses), and the multiple sources of resistance will permit the eventual construction of resistance gene pyramids that should provide durable resistance to SBR.

- *Developing protection to wheat and barley stripe rust.* Stripe rust continues to cause chronic losses in U.S. wheat and barley grain production. In 2008, ARS scientists in Pullman, Washington, evaluated more than 15,000 wheat and 5,000 barley breeding lines for stripe rust resistance. Results were provided to U.S. wheat and barley breeders to accelerate national efforts to reduce stripe rust losses to U.S. grain production. Assessment in FY 2008 and previous years resulted in the release in 2008 of five new wheat varieties with stripe rust resistance.
- *Development of DNA markers for breeding for wheat and barley protection from scab.* Robust DNA markers are needed to accelerate resistance breeding for the major wheat and barley disease, *Fusarium* head blight (FHB). ARS researchers at Fargo, North Dakota, in collaboration with researchers at the University of Minnesota, developed robust co-dominant DNA markers from candidate gene region controlling scab resistance from the resistant line, Sumai 3. These markers were validated and used to screen breeding lines submitted by wheat breeders in the Northern Plains region. These markers will expedite the identification and selection of desirable alleles for FHB resistance in regional wheat breeding programs. ARS researchers at Manhattan, Kansas, have characterized the wheat landrace (Wangshuibai), which has additional genetic resistance to *Fusarium* and have initiated marker development. ARS researchers in Raleigh, North Carolina, determined that wet weather later in the grain-fill period encourages mycotoxin development resulting from scab. They have demonstrated that increasing numbers of moist days in the post-flowering period are associated with elevated disease and mycotoxin accumulation. These findings will help breeders screen for resistance to mycotoxin development during wet springs and provide more accurate forecasts of mycotoxin levels.
- *The Sclerotinia Initiative.* *Sclerotinia sclerotiorum* causes white mold on more than 400 plant species including many economically important crops like beans, canola, soybean, sunflowers, peas, lentils and chickpeas. A team of ARS scientists at several locations in the United States have research accomplishments that range from Integrated Pest Management (IPM), disease warning systems, breeding programs for resistance development, and molecular strategies for elucidating mechanisms that cause disease in their hosts. A few of the substantial advances are included here as contributions of the Sclerotinia Initiative:
  - *Sunflower resistance development:* The two most important diseases affecting U.S. sunflowers are stalk rot and head rot caused by *Sclerotinia sclerotiorum*. None of the available sunflower hybrids have adequate resistance to *Sclerotinia*. In attempts to achieve higher levels of resistance, wild progenitors were screened for different and potentially unique genes. Of 150 accessions identified in previous tests with resistance superior to those of the best commercial hybrids, 20 have been selected that show promise for resistance in greenhouse trials. In addition to conventional breeding, advances have been made in sunflower transformation to speed improved *Sclerotinia*

resistance development. A confection sunflower line was identified as exceptionally responsive to tissue culture manipulation, and successful shoot production from pre-germinated cotyledonary tissues was demonstrated. Cotyledonary tissues of sunflower have also been shown to be highly responsive to *Agrobacterium*-mediated transformation. Through improved screening methods of misting after inoculation, pyramiding new genes with downy mildew and rust resistance genes, and new transformation methodology, *Sclerotinia* resistance is achievable in sunflower.

- *Legume resistance*: Pinto beans are the most widely grown commercial class of dry beans in the United States and are among the most susceptible to white mold. Novel QTL resistance was identified associated with white mold resistance from undomesticated wild bean germplasm in a population of inbred backcross lines (IBL). Identification of QTL for white mold resistance in a well adapted pinto background suitable for direct harvest will serve as a valuable bridge to develop resistance in other medium-seeded classes and further our understanding of white mold resistance in dry beans. Natural genetic resistance is also limiting in pea germplasm. Both conventional breeding and genetic transformation methods have been used to develop resistance in pea that will deliver a product with high consumer acceptance. Varieties and germplasm accessions with improved resistance to *Sclerotinia* white mold have been identified.

#### **Component IV – Biological and Cultural Strategies for Sustainable Disease Management**

- *Biocontrol of cacao pathogens evaluated*. Diseases of cacao caused by the fungus *Moniliophthora* severely reduce yields in Central and South America. Antagonistic fungal species in the genus *Trichoderma* with potential for biologically controlling these diseases were collected over the past several years from cacao environments in Ecuador. Lab and growth chamber experiments were designed and carried out characterizing the parasitic activity of the *Trichoderma* isolates against *Moniliophthora roreri* and *Moniliophthora perniciosa*, major pathogens of cacao. In addition, tests were carried out characterizing the ability of each beneficial fungal species to become established in the field. The completion of this research has allowed rapid movement of the associated research into the field where 14 isolates have been selected for small scale field screening in Ecuador and in Costa Rica. The selected isolates have significant biocontrol activity against pathogens of cacao and have potential to increase yield where disease pressure can be severe. In addition, these isolates are being evaluated, with some success, for biocontrol activity against pathogens of crops grown in the United States. Another important disease of cacao in Cameroon is black pod, which seriously damages cacao, with greatly decreased production. The fungus *Trichoderma* also has been shown to successfully control black pod disease. Specific strains of *Trichoderma* were determined to be especially effective in controlling this disease. Plant pathologists will use these findings to develop non-chemical methods for controlling black pod disease of cacao.

- *Mechanisms of suppression of fire blight of apple by new biocontrol agent.* Fire blight, a serious disease of apple and pear caused by *Erwinia amylovora* has been controlled in the past mainly with antibiotics, but, as with human diseases, bacterial resistance to these antibiotics has necessitated alternative strategies such as biological control. One strain of biocontrol agent, *Pantoea agglomerans*, was identified through ARS research at Wenatchee, Washington, and was recently made available commercially. An explanation of why its strain is a superior antagonist of *E. amylovora* on flower stigmas, where the two organisms compete for establishment, has been elusive. Recent studies indicate that in addition to competing for nutrients, this strain may also suppress *E. amylovora* by lowering the pH to levels less conducive for pathogen growth and produce a unique antibacterial compound highly specific to the pathogen and active under acidic conditions. This information and additional studies will likely lead to improvements in fire blight management with biological agents.
- *Xylella fastidiosa colonizes plant xylem by both active and passive mechanisms.* *Xylella fastidiosa* (*Xf*) is a bacterial pathogen that causes numerous devastating plant diseases, including Pierce's disease of grape, but the exact mechanism of disruption and systemic colonization of host xylem tissues remains elusive. Data collected by ARS scientists at Davis, California, suggest that the bacteria induce embolism, via pit membrane degradation, in the early stages of colonization, and this is the first step leading to eventual vessel plugging. Measurements of pit membrane porosity showed that pit membrane pores larger than individual *Xf* cells occur frequently throughout the secondary xylem and likely facilitate systemic colonization in susceptible oak species. This work provides a better understanding of how this pathogen induces symptoms and moves within its hosts, and may be used to select for structural characteristics in breeding programs that impart resistance.
- *Discovery of a novel insecticidal toxin from the genomic sequence of Pseudomonas fluorescens Pf-5.* Genomic sequencing of microorganisms has revealed a tremendous source of biologically-active compounds of potential value for pest management. In collaboration with colleagues in Switzerland, ARS scientists in Corvallis, Oregon, identified a new insect toxin in the genome of the bacterial biological control agent *Pseudomonas fluorescens* Pf-5. They also demonstrated that the toxin is active against tobacco hornworm and waxworm larvae, and a manuscript has been published describing this finding. This research demonstrated the power of a genomic approach for the discovery of valuable natural products.
- *The Sclerotinia Initiative.* *Sclerotinia sclerotiorum* causes white mold on more than 400 plant species including many economically important crops like beans, canola, soybean, sunflowers, peas, lentils and chickpeas. A team of ARS scientists at several locations in the United States have research accomplishments that range from Integrated Pest Management (IPM), disease warning systems, breeding programs for resistance development, and molecular strategies for elucidation of the mechanisms

that cause disease in their hosts. A few of the substantial advances are included here and in Component III as contributions of the Sclerotinia Initiative:

- As part of the Sclerotinia Initiative, various IPM strategies have been proposed to the dry bean (and other crop) industries for management of white mold, but adoption has been slow. ARS scientists have demonstrated the effectiveness of individual IPM components such as fungicides, cultural practices, and cultivar selection, including the incorporation of advanced breeding lines developed by two germplasm improvement projects, for collectively managing this important pathogen in the central U.S. High Plains and adjacent regions of North America. Assuming modest disease control and associated yield gain with an upright, susceptible cultivar like Vision, one fungicide application, and two lines per bed, a grower could net an additional \$190/acre or \$512/hectare at a cost of \$30/acre or \$74/hectare for the fungicide application in the presence of white mold. Results from this project will support the development of improved germplasm, modified production practices, and effective integrated pest management strategies against white mold in bean production throughout the United States.
- Very little is known about how the fungus, *Sclerotinia sclerotiorum*, causes disease in plants. This has been attributed to a lack of an efficient genetic engineering system for functional analyses of genes. With the genome of *S. sclerotiorum* being sequenced, development of an efficient transformation system was developed to efficiently transform *S. sclerotiorum* through Agrobacterium-mediated transformation. ARS scientists generated 137 stable genetic transformed cultures, and identified 17 with potential genes that are responsible for disease. Four putative genes of known pathogenicity factors were identified in selected transformants. These putative genes need to be confirmed for their roles in pathogenicity. Completion of the research will determine and demonstrate the functions of the identified putative genes in pathogenicity of *S. sclerotiorum*. Results of the research will enhance understanding of pathogenic mechanisms of *S. sclerotiorum*, providing better knowledge for devising disease control strategies such as novel approaches to engineering resistant crops.