

USDA  
AGRICULTURAL RESEARCH SERVICE

**NATIONAL PROGRAM 303 – PLANT DISEASES**  
**ANNUAL REPORT FY 2007**



## National Program 303 – PLANT DISEASES FY 2007 Annual Report

### *Introduction*

The overall goal of National Program 303 – Plant Diseases – 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.

This National 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. 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.

National Program 303 is comprised of 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 2007, this program produced several important discoveries and advances. Some of these are described below, grouped by program component:

### **Component 1: Disease Diagnosis: Detection, Identification and Characterization of Plant Pathogens**

*Squash vein yellowing virus (SqVYV)*, a novel pathogen was shown to be the cause of watermelon vine decline by ARS scientists at Fort Pierce, Florida. Improved diagnostics for detection of SqVYV were developed during the current year's research. This technology has been transferred to state regulatory and university diagnostic laboratories

to facilitate SqVYV detection, and was used by ARS in the first report of SqVYV outside of Florida (in Indiana).

By the time Botrytis infections of grape are detected visually, fruit quality has already suffered. ARS scientists in Geneva, New York, developed an early detection technology based on quantitative PCR to detect and monitor Botrytis infections up to 2 months prior to visual detection. This ability to detect fungal infection prior to damage and secondary spread will enable growers to reduce the frequency of fungicide applications and specifically target applications when needed.

Pierce's disease (PD) of grape is one of the major threats to the Nation's grape and wine-dependent industries. ARS scientists at Parlier, California, discovered a new plasmid of the bacterium *Xylella fastidiosa* (Xf), which is responsible for the disease, that may be useful in transferring genes of interest into the bacterium. The unexpected relationship of the Xf plasmid with a plasmid from an unrelated bacterium inhabiting a completely different ecological niche (a bacterial symbiont of earthworms) suggests that Xf may exchange DNA with a much wider array of microbes than previously realized. Also, ARS researchers: (1) Developed an electrical penetration graph for sensing xylem penetration by feeding the glassy-winged sharpshooters, the vector of Pierce's disease; (2) Sequenced the genomes of two new almond leaf Xf scorch strains and compared them to other strains of Xf, revealing new information about virus integration in the bacterial chromosome that will be useful for understanding evolution and host range of the bacteria; (3) Used SNPs to develop an unambiguous system for identification of the causative bacterium; and (4) Identified nine genes involved in signal transduction and defense responses from resistant grapes, useful for marker-assisted breeding. These discoveries will be used to design strategies for interfering with transmission of the bacterium and on developing control strategies.

Currently no rapid, sensitive, and cost effective detection technologies exist for *Brenneria rubrifaciens*, the causative agent of deep bark canker of walnut. Three species specific primer sets were developed by ARS scientists at Davis, California. These primer sets were used to establish parameters for detection of *Brenneria* from sap and from soil. A semi-selective medium for *B. rubrifaciens* isolation from environmental samples was also developed. These detection methods will be used to develop effective control strategies for both nursery and orchard conditions.

An innovative PCR-based genome walking approach was developed by scientists in Parlier, California, to identify genomic DNA sequences of *Candidatus Liberibacter*, the pathogen that causes Huanglongbing disease in citrus. The identified sequences will help scientists develop new genome-based detection tools for the bacterium.

A new wheat stem rust mutant, called Ug99, has appeared in East Africa that threatens global production. Virulence of the disease-causing ability of stem rust strains collected in East Africa has been assessed. ARS researchers at the Cereal Disease Laboratory, St. Paul, Minnesota, determined that the Ug99 rust mutant can now overcome the wheat resistance gene, Sr24. The detection of Sr24 virulence is significant because this

resistance gene is widely used in breeding of U.S. wheat, as well as many other major wheat production regions. Accurate assessment of Ug99 virulence is critical for cereal breeders who are developing genetic resistance to the new African stem rust mutant.

Huanglongbing (HLB), also known as citrus greening, is the most devastating citrus disease, and is now wide-spread in Florida. The disease also is imposing an immediate threat to the citrus industry in California and Texas. Because of the fastidious nature of HLB bacteria (*Candidatus Liberibacter asiaticus*), a high-throughput detection technology for HLB is critical for HLB research and disease management. Polyclonal antibodies against HLB pathogen have been developed by researchers at Fort Pierce, Florida, and a series of primers and probes were also developed for better detection of HLB using PCR or *in situ* hybridization. These technologies have been transferred to university and state regulatory research laboratories for HLB detection and other research purposes.

Reports from growers of unusual rust symptoms in the South Florida sugarcane growing area in the spring of 2007 turned out to be Sugarcane Orange Rust, caused by *Puccinia kuehnii*. This is the first reported incidence of this disease in the Western Hemisphere. Although its impact in Florida is unknown, in Australia, the orange rust caused substantial yield losses in 2000. ARS scientists in Florida and Louisiana who develop new high-yielding sugarcane cultivars are now evaluating cultivars that are resistant to orange rust as well as several other major diseases.

## **Component 2: Biology, Ecology, Epidemiology, and Spread of Plant Pathogens and Their Relationships with Hosts and Vectors.**

Soybean rust, caused by *Phakopsora pachyrhizi*, is a devastating foliar disease of soybeans that has spread from Asia to Africa, South America, and the United States. Available genomic sequence data were mined by ARS scientists at Frederick, Maryland, for simple sequence repeats, called microsatellites, to develop a set of molecular markers which could be used to distinguish individual isolates of the pathogen. Twenty-eight *P. pachyrhizi* isolates from 13 countries were surveyed using 24 microsatellite markers. Nineteen of these microsatellite markers were useful for assessing genetic variation and for distinguishing the isolates. Six *P. pachyrhizi* isolates collected from the initial outbreak of soybean rust in Louisiana and Alabama in November 2004 were included in the study and were shown to be genetically diverse. These six U.S. isolates also showed different levels of virulence when inoculated onto soybean accessions containing the four rust resistance genes Rpp1-4. The microsatellite markers developed from this project can detect differences between *P. pachyrhizi* isolates and will be useful for population and genetic studies on this economically important pathogen.

Plant pathogenesis is a complex process involving the coordinated expression of many genes in response to environmental signals associated with interactions with the host. ARS scientists at Ithaca, New York completed the first characterization of the primary regulatory mechanism underlying the expression of the type III secretion system and associated protein effectors in DC3000, a pathovar of *Pseudomonas syringae*. In

particular, the identification of genes encoding effector proteins secreted into the plant has enabled other groups to make rapid progress in understanding the molecular interactions underlying pathogenesis and plant defense responses.

There are numerous genotypes of soybean cyst nematode (SCN), and no soybean germplasm is resistant to all genotypes. ARS researchers at Beltsville, Maryland, determined which soybean genes are expressed in resistant roots compared to susceptible soybean roots at 12-hours, 3-days, and 8-days after inoculation of roots with soybean cyst nematode by surveying expression of 35,000 soybean genes. A subset of fewer than 100 genes was identified that are highly expressed in resistant roots. These genes are promising candidates for gene transfer experiments to determine if they will broaden resistance of soybean to SCN. These candidate genes may provide significant resistance and increase soybean yield for farmers.

Huanglongbing (HLB), also known as citrus greening, is the most serious, insect-transmitted disease of citrus, and has become established in Florida. Control of citrus psyllid vector populations is a vital component of disease management. ARS scientists at Frederick, Maryland, determined the effect of imidacloprid insecticide on transmission of HLB. Following incorporation of the insecticide into sweet orange seedlings, infectious psyllids were aspirated on the seedlings and monitored for survival and disease transmission. Although most psyllids died within the first 24 hours, strong symptoms of HLB infection were evident by 8 weeks in most plants, indicating the insects could transmit the causal HLB bacterium before dying.

USDA Animal and Plant Health Inspection Service (APHIS) and regulatory agencies in California and Texas are concerned about canker-infected fruit as a pathway for canker infection to new citrus production areas. Survival studies with canker-infected and non-infected fruit combinations were conducted in Florida, Argentina, and Brazil, by scientists at Fort Pierce, Florida, to examine survival characteristics. In all three locations, a few bacteria were recoverable after packinghouse processing and decontamination treatment. Post-processed fruit in discarded cull piles did not function as a source of infection for susceptible young grapefruit trees located 1, 5, and 10 meters from the piles. These findings are in concurrence with the APHIS Pest Risk Assessment and Risk Management Analysis on 'Movement of Commercially Packed Citrus Fruit from Citrus Canker Disease Quarantine Areas', and thus may have impact on interstate and international shipment of citrus fruit.

### **Component 3: Plant Disease Resistance.**

The structurally conserved products of plant disease resistance genes can collectively mediate resistance to a diverse range of pathogens and suggest that plants may have evolved common resistance mechanisms. Scientists in the ARS Plant Gene Expression Center, Albany, California, used classical genetic and reverse genetic-virus induced gene-silencing (VIGS) approaches and identified several genes encoding components of the resistance signaling pathway. A caspase-like protease and a predicted lipase-domain containing protein were also identified. These experiments will lead to an understanding

of how a limited number of structural classes of resistance (R) proteins cope with the potentially large array of diverse pathogens encountered by plants.

The infection process of the Fusarium head blight (FHB) pathogen (caused by *Fusarium graminearum*) on wheat heads has been studied under conditions of artificial inoculation and controlled conditions. However, little is known regarding whether these results accurately reflect the field infection process. Colonization of wheat heads by an FHB antagonist patented by ARS scientists at Peoria, Illinois, *Cryptococcus flavescens* OH 182.9, under field conditions is also poorly understood. In a 2-year collaborative study between researchers in Peoria and Wooster, irrigated, field-grown wheat, infected by *F. graminearum* naturally released within the field, was studied using scanning electron microscopy to demonstrate that germ tubes from ascospores can enter stomates of the wheat head glume and lemma tissues within 3 days of flowering. On irrigated and non-irrigated wheat grown under field conditions, the populations of *C. flavescens* increased several orders of magnitude or recovered dramatically on wheat heads after natural rainfall events, respectively, indicating the high level of colonization by the antagonist. These results clarify the infection process of the *F. graminearum* under field conditions and will assist the directed development of formulations that improved the effectiveness and consistency of biocontrol agents (*C. flavescens*) active against the pathogen.

Phytophthora root rot (PRR) is a soilborne disease that primarily attacks the roots of soybean plants. Partial resistance and tolerance can be used to augment complete resistance to PRR. Partial resistance and tolerance to two compatible races of *Phytophthora sojae* were identified by scientists at Urbana, Illinois, in private commercial soybean cultivars possessing the complete resistance gene Rps1k. Many of these cultivars had a high level of partial resistance in the greenhouse and in the field. Most of them appeared to be consistently tolerant to Phytophthora root rot in the field based on yields of plants in inoculated and non-inoculated plots. This information is useful to commercial seed companies, soybean growers, and consultants that recommend cultivars with resistance to growers or develop resistant cultivars.

The fungus *Stagonospora nodorum* is the causal agent of nodorum blotch of wheat. New discoveries by ARS researchers at Fargo, North Dakota, are unraveling the processes used by the fungus to damage wheat. Results reveal that the fungus produces host-selective toxins which correspond to single sensitivity genes in wheat. One novel toxin, called SnTox2, has been shown to interact either directly or indirectly with a specific wheat gene, and this process accounts for as much as 58 percent of the disease phenotype. This research is defining the disease mechanism and results will be used to develop new wheat varieties with better protection for the blotch pathogen.

Yellow dwarf virus disease is caused by a collection of viruses, one of which is *Cereal yellow dwarf virus* (CYDV). ARS researchers at West Lafayette, Indiana have derived resistance to this virus from wheatgrass, a grassy relative of wheat. Working in partnership with Purdue University researchers, they have integrated a very high level of resistance to CYDV into commercial wheat varieties. Recent results have proven that

this resistance is primarily due to a block in movement and also a reduced feeding ability of the aphid insect that carries this virus from plant to plant. This combined resistance to the virus and the insect vector is extremely effective in the field. This resistance is now being used by wheat breeders worldwide because it is so effective and potentially durable.

The soybean aphid is one of the emerging threats to soybean production in the United States. Severe aphid infestations can reduce soybean seed yield directly and can transmit certain plant viruses such as *Alfalfa mosaic virus*, *Soybean dwarf virus*, and *Soybean mosaic virus* to soybean. In 2006, ARS scientists at Wooster, Ohio, identified three soybean plant lines with resistance to the soybean aphid. Mapping populations were developed using these sources of resistance. Using simple sequence repeat markers, a gene for aphid resistance was mapped that is different from the aphid resistance genes from two of the cultivars. This new gene is especially important, because unlike the genes from these two lines, it provides resistance against both known biotypes of soybean aphids. This gene can be used for developing soybean cultivars that will provide broad resistance against the soybean aphids.

Resistant varieties of soybean will minimize the yield losses to growers by soybean cyst nematode. A new soybean germplasm line is under development by scientists at Stoneville, Mississippi, and is being tested for its performance. This line, JTN-5203, was ranked second among 22 entries evaluated in USDA Southern Regional Uniform Group V Tests, and yielded nearly 50 bushels per acre. This line is highly resistant to nematode race 14, and is also resistant to stem canker and moderately resistant to frogeye leaf spot. It will be further evaluated for other nematode populations with a consideration for release as a germplasm.

Stripe rust epidemics have caused major losses for the wheat and barley producers and industry in recent years in the Pacific Northwest, Midwest, and Texas. ARS researchers at Pullman, Washington, monitored the severity and distribution of stripe rust strains in the United States and provided disease forecasts. More than 17,000 wheat and barley breeding lines were evaluated for stripe rust resistance nationally and a new core collection of wheat and barley germplasm with rust resistance was provided to U.S. breeding programs. A gene for high-temperature, adult-plant resistance was genetically mapped and a molecular marker identified. These research results will accelerate the development of wheat and barley cultivars with stripe rust protection and are helping growers minimize damage from the disease through efficient management and economical use of fungicides.

Anthracnose is a major fungal disease of sorghum in the United States. ARS researchers at College Station, Texas, and Mayaguez, Puerto Rico, have evaluated 19 sorghum accessions obtained from Uganda and demonstrated that several exhibit high resistance to anthracnose. The anthracnose-resistant sorghum germplasm can be exploited by breeders to develop resistant sorghum varieties that will enhance production efficiency and profitability.

#### **Component 4: Biological and Cultural Strategies for Sustainable Disease Management**

Powdery mildew of hop is an economically limiting disease for producers. Fungicides commonly used for powdery mildew management increases populations of and damage by spider mites in numerous crops including hop, leading to increased use of pesticides. ARS scientists at Corvallis, Oregon, in collaboration with Washington State University researchers, identified powdery mildew management programs that enhance biological control of spider mite by natural enemies. This discovery may allow growers to minimize pesticide use by enhancing the reliability of conservation biological control of spider mites, and result in optimized fungicide use, reduced input costs for producers, and enhanced environmental protection.

Tropical spiderwort is an invasive weed which typically reaches very high plant population densities in cotton, corn, and peanut fields in some parts of the coastal plain of the Southeastern United States. Many weeds can support nematode reproduction and plant-pathogenic fungi, thereby reducing the effectiveness of crop rotation as a management tool. ARS scientists in Tifton, Georgia, showed that tropical spiderwort supported significant levels of reproduction by two wide-spread species of root-knot nematodes (southern root-knot and peanut root-knot), the reniform nematode, and a major peanut disease (southern stem rot). At the high plant densities often found in fields, tropical spiderwort is a sufficiently good host for some of the primary nematode and fungal pathogens of major crops in the southeastern United States that will greatly reduce the pathogen-suppressive effects of crop rotation. Therefore, suppressing tropical spiderwort is an important component of suppressing these pathogens.

Conventional chemicals used to control the root-knot nematode (*Meloidogyne incognita*) may be deleterious to the environment and to non-target organisms. In lab assays, plant parasitic nematodes were placed in clove oil, exposed to volatile compounds from the oil, and placed in soil treated with clove oil. Concentrations of clove oil active against the nematodes were tested in the greenhouse for toxicity to plants and for suppression of nematode population on plant roots. ARS scientists in Beltsville, Maryland, demonstrated that the tested formulation was active against nematodes, and that phytotoxicity needs to be taken into account.

The presence of riparian areas adjacent to North-Coastal California vineyards contributes to Pierce's disease, as evidenced by a correlation between disease incidence and proximity of vines to riparian areas, but riparian hosts of the pathogen (*Xylella fastidiosa*) have not been verified by recovery of the pathogen from field plants. The research performed by ARS scientists in Davis, California, found that only one riparian host, *Vinca major* (periwinkle), was positively correlated with a high incidence of Pierce's disease in adjacent vineyards. Based on these results, eradication of all riparian hosts may be unnecessarily costly, and overly disruptive to riparian ecosystems. Research will aid farmers and administrators in developing effective pest management techniques, particularly in north coastal California vineyards.



Genomic sequencing of microorganisms has revealed a tremendous source of new metabolites that could serve as novel antibiotics and fungicides that could be valuable for agriculture or human health. From the genome sequence of *Pseudomonas fluorescens* Pf-5, ARS scientists at Corvallis, Oregon, identified three new gene clusters and three novel metabolites that are toxic to fungi or insects. The structure and bioactivity of one of these metabolites (orfamide A), the founding member of a new class of cyclic lipopeptides, was published this year. Corvallis researchers also discovered that five derivatives of the anti-fungal compound Rhizoxin, including one novel compound, are produced by Pf-5. These compounds are toxic to two important plant pathogens – *Botrytis cinerea* and *Phytophthora ramorum*. This research demonstrates the power of a combined genomic and chemical approach for the discovery of valuable natural products.

Scientists at ARS Wenatchee, Washington, and Oregon State University evaluated the potential for co-occurrence of the fire blight bacterium, *Erwinia amylovora*, with mature symptomless winter pear fruit. *E. amylovora* shows similar survival characteristics on both pear and apple fruit. The disease organism does not exist inside mature symptomless pear fruit and its presence is exceptionally rare on commercially produced fruit. Survival of *E. amylovora* on fruit surfaces is unlikely during post-harvest chilling given the high population size required for persistence. This information will be of value in efforts to expand foreign markets for U.S. pears.