

Plant Diseases FY 2004 National Program Annual Report

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Introduction

This program addresses the characterization of plant diseases and strategies to control them. The program is divided into five components: identification and classification of pathogens; biological control; cultural control; pathogen biology, genetics, population dynamics, spread, and relationship with hosts and vectors; and host plant resistance to disease. 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 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.

Selected Accomplishments by Component

Component I. Identification and Classification of Pathogens

New Disease of Pears Identified. In 2001, 2002, and 2003, decay caused by an unknown pathogen and therefore with unknown risk to U.S. pear production developed on Ya-Li pears imported from China during marketing in the United States. Studies were conducted by ARS scientists in Wenatchee, Washington, to identify the causal organism(s). The results indicated the causal organisms were members of the *Alternaria* group of fungi not known to exist in the United States. This information has been used by USDA-APHIS as the basis for suspension of the Ya-Li pear importation program until such time that further information can be developed to establish the risk to U.S. agriculture from further imports of this fruit, or an importation protocol can be defined that prevents importation of infected fruit.

Molecular Biomarkers Identified. Phytoplasmas and spiroplasmas cause many agriculturally important diseases of plants, but the development of effective disease control measures is hampered by difficulties in identifying the pathogen strains and species, and by lack of knowledge about how phytoplasmas and spiroplasmas survive and cause diseases. ARS scientists working with other U.S. scientists and foreign collaborators identified candidate molecular biomarkers that can distinguish strains and species of these pathogens and that are of potential significance in the survival of the

pathogens in their hosts and in the development of plant diseases. They also developed genetically engineered plants that are capable of synthesizing a foreign protein in specific tissues where the spiroplasma and phytoplasmas live in diseased plants, anticipating future delivery of anti-spiroplasma and anti-phytoplasma proteins to these tissues. This accomplishment provides scientists, plant disease diagnosticians, and plant quarantine officials' new tools for controlling plant diseases caused by spiroplasmas and phytoplasmas.

Relationships of Bunt Fungi on Cereal Crops Determined. A bunt fungus called *Neovossia iowensis* was thought to be a close relative of the Karnal bunt and rice kernel smut fungi. Research showed that the *Neovossia* bunt fungus is more closely related to wild-grass infecting *Tilletia* species than to Karnal bunt or rice kernel smut. This research also confirms that Karnal bunt and rice kernel smut are true bunt fungi in the genus *Tilletia*. This information will be used by plant pathologists to correctly identify these disease-causing fungi. In addition, this research will ensure that plant quarantine policy is effective at preventing the entry of fungi that most directly threaten U.S. agriculture. An interactive key for the identification of teliospores of bunt fungi in the United States is available over the Web at <http://nt.ars-grin.gov/TaxaDescriptions/Tilletia/Index2.cfm>.

A Monograph of 30 Species of Green-Spored *Hypocrea* and Their *Trichoderma* Asexual States was Completed And Published. This group includes many species of *Trichoderma* that are used in biological control. Species descriptions are based on morphological characteristics with connections made between the *Hypocrea* sexual state and the *Trichoderma* asexual state. In addition, phylogenetic analyses of two genes for each species are included. Descriptions, illustrations and keys allow plant pathologists to identify the isolates that they are testing for use in the biological control of plant pathogens.

Reclassification of Citrus Canker Pathogen. Efforts to eradicate citrus canker are currently costing the citrus industry and the state of Florida millions of dollars. The true citrus canker disease is difficult to diagnose, due to the presence of other forms of the disease which are not regulated and which are caused by closely related bacteria. ARS scientists at Frederick, Maryland, have proposed the reclassification of the true citrus canker pathogen, *Xanthomonas campestris* pv. *citri* (XCC) as *X. smithii* subsp. *citri* (XSC), and designed a molecular diagnostic assay specific to the causal organism. The utility of the assay was demonstrated by detecting the pathogen in imported dry citrus leaves at two California ports. Having a reliable PCR assay available for specific detection of XSC in citrus leaves will help prevent the inadvertent reintroduction of this dangerous pathogen into the United States.

Characterization of New Root-knot Nematode on Peach. An international collaborative project pertaining to a root-knot nematode previously misidentified and known to parasitize Guardian rootstock was completed. The new species was named the Peach Root-Knot Nematode, *Meloidogyne floridensis*. These findings are important because they will enable extension personnel and scientists to differentiate this nematode from

other species. Collaborators of this project included ARS (Byron & Beltsville), France (INRA), and The Netherlands (PRI).

New Races of Root Rot Pathogen Identified. Soybean yield losses due to the root rot pathogen, *Phytophthora sojae* are significant under certain conditions. ARS scientists at West Lafayette, Indiana, evaluated plant and soil samples from Indiana soybean fields and compared them to previous years data to identify races of this genetically diverse pathogen. Results of current research now identify 12 predominant races and verify a need for gene combinations or new genes to control *Phytophthora* root rot. Virulence data suggest new gene combinations will control races identified in soybean fields, whereas disease control using other resistance genes would be less than 50%. This type of research provides new insights about genetic diversity and distribution of *P. sojae* that will provide clues to key factors influencing change in this important pathogen and it is impacting development of resistant soybean cultivars, which should result in significant economic benefit.

Better Identification of a Serious Citrus Disease. Citrus tristeza virus (CTV) is responsible for two field diseases of citrus, both of which have caused massive losses to citrus industries in Asia, South America and Africa. ARS scientists at Fort Pierce, Florida, demonstrated the utility of using degenerate primers and PCR to identify genetically distinct components in complex mixtures of citrus tristeza virus. This enhances regulatory measures concerning identification and control of citrus tristeza virus. Research at Fort Pierce also provided identification of aphid transmissible and non-transmissible CTV isolates as a baseline for studying viral genetic control of CTV transmission by aphids. These findings will greatly enhance our ability to diagnose and identify this potentially serious disease and allow for more rapid control responses.

Component II. Biological Control

Biological Control of Fusarium Head Blight. Fusarium Head Blight (FHB), or scab, is the most serious disease of wheat in the United States. A key impediment to the commercialization of biocontrol agents is the lack of knowledge of cultivation and formulation technologies needed to maintain their efficacy via maximizing the tolerance of stresses encountered during cultivation, separation, processing and storage. ARS scientists in Peoria, Illinois, developed cultivation and formulation technologies that now make it possible to develop a more effective, air-drying-tolerant product, thereby enhancing the possibility of producing a commercially available FHB biocontrol product.

Armillaria Root Disease Control. *Armillaria mellea* is very difficult to eradicate from vineyard and orchard soils, even with soil fumigants such as methyl-bromide. ARS scientists developed an effective, inexpensive, cultural practice known as 'root collar excavation' for control of *Armillaria* root disease of grapevines, a disease for which no other post infection control practices exist on any of the 500+ hosts of the causal pathogen, *Armillaria mellea*, worldwide. A field experiment testing the efficacy of root collar excavation was established in California in two commercial vineyards in Napa County, California. Excavation significantly increased yield and cluster weight of

symptomatic grapevines in one of two examined vineyards and *Armillaria mellea* mycelial fans receded from root collars of symptomatic-excavated grapevines, but remained on root collars of symptomatic-nonexcavated grapevines. Root collar excavation appears to be a promising approach for control of *Armillaria* root disease, as long as excavated root collars are kept clear of soil.

Component III. Cultural Control

Alternative Measures for Postharvest Decay. As an alternative to chemical control to reduce losses due to postharvest decay, ARS scientists at Beltsville, Maryland, in cooperation with ARS scientists at Kearneysville, West Virginia, combined heat treatment, sodium bicarbonate, and two biocontrol agents, alone or combined to reduce postharvest fungal decay of apples. Both bitter rot and blue mold decays were effectively controlled by a combination of either of the antagonists and heat treatment. In nonheated fruit, the combination of the two biocontrol agents plus sodium bicarbonate resulted in the smallest lesions on fruit decayed by the blue mold fungus. This control strategy could potentially be used commercially to reduce our dependency on fungicides.

Common Mycorrhizal Networks Play an Important Role in Nutrient Uptake and Transfer from No-till Cover Crops to Grapevines. Roots of both grapevines and cover crops form mutualistic symbioses with arbuscular mycorrhizal (AM) fungi, and may be interconnected by AM hyphae. To study nutrient transfer from cover crops to grapevines through AM fungal links, ARS scientists grew grapevines and cover crops in specially-designed containers in the greenhouse that restricted their root systems to separate compartments but allowed AM fungi to colonize both root systems, and labeled leaves of two cover crops, a grass (*Bromus hordeaceus*) and a legume (*Medicago polymorpha*), with ¹⁵N for 24 h. Results indicate that AM fungi-mediated ¹⁵N transfer from cover crops to grapevines five and ten days after labeling and that N transfer was significantly greater from the grass to the grapevine than from the legume to the grapevine, suggesting that grasses are better N donors than legumes. Results of this research show that no-till cover crops may enhance nutrient uptake in vineyards, which may encourage more grape-growers to maintain no-till cover crops.

Pierce's Disease, Caused by *Xylella fastidiosa*, is the Most Significant Disease of Grapes in the Western United States. To examine the effects of seasonal temperature changes on *X. fastidiosa* populations in riparian hosts, ARS scientists quantified *X. fastidiosa* on a seasonal basis in 50 replicates each of five species of potted, infected, riparian plants in two locations: a relatively cool area (Mendocino County, California) and a warmer area (Napa County, California). It was determined that *Xylella fastidiosa* populations were significantly higher in Napa County than in Mendocino County, suggesting that the historically higher incidence of Pierce's Disease in Napa County may be due, in part, to its higher annual temperatures. It was further determined that early season infections of grapevines with Pierce's Disease in the North Coast of California are caused by overwintering blue-green sharpshooters (*Graphocephala atropunctata*) that acquire the Pierce's Disease pathogen from riparian hosts in autumn instead of in spring, as was previously believed. Based on low *X. fastidiosa* populations detected in winter and spring

in riparian hosts, it appears that overwintering hosts may not be as important as originally thought; instead, infective adult *G. atropunctata* that overwinter may be more important in initiating spring infections of grapevines.

Preplant Rotation as Good as Methyl Bromide Fumigation. Selected crop rotations in conjunction with Guardian rootstock were evaluated as a potential preplant ground cover management strategy in suppressing the ring nematode population density and to determine the influence long-term preplant crop rotations have on peach tree growth and incidence of Peach Tree Short Life (PTSL). Results indicate that nematode populations were greater on Nemaguard than for Guardian rootstock on all sampling dates (2000-2003), indicating that Nemaguard was a better host for the nematode. Preplanting 3-continuous years of canola/sorghum was as effective as preplant methyl bromide fumigation in increasing tree survival from PTSL 26 months after establishing the orchard. These data provide useful insights into the use of canola/sorghum in conjunction with Guardian rootstock as a pre- and postplant IPM alternative to chemical control for managing ring nematode on PTSL sites in the Southeast.

Compatible Winter Cover Crops in Cotton Production Systems. There is growing interest in planting legumes as winter cover crops in cotton production systems; however, these legumes may support reproduction of the southern root-knot nematode which is a serious pest of cotton. Experiments were conducted by ARS scientists in Tifton, Georgia, to determine the amount of nematode reproduction on legume cover crops (vetches and clovers) and subsequent damage to cotton. Of the legumes tested, only Cahaba white vetch and Cherokee red clover were resistant to nematode reproduction and did not lead to damaging nematode populations in the subsequent cotton crop. Planting Cahaba white vetch or Cherokee red clover should have a lower risk of building up damaging populations of the southern root-knot nematode than other nematode-susceptible legume crops.

Determination of Usefulness of Double Cropping Cucumbers and Squash After Resistant Bell Pepper to Manage Root-Knot Nematodes. Primary control for root-knot nematodes in susceptible vegetable crops is pre-plant fumigation of the soil with methyl bromide; however, methyl bromide is scheduled for removal from the U.S. market in 2005. Rotation of resistant and susceptible crops could be used as an alternative to methyl bromide to manage root-knot nematodes. Field tests were conducted in South Carolina and Georgia to study efficacy of planting root-knot susceptible vegetable crops (cucumbers and squash) after the root-knot nematode resistant bell pepper "Charleston Belle" in fields that were highly infested with root-knot nematodes. "Charleston Belle" reduced nematode numbers in the soil and increased yields of nematode-susceptible cucumber and squash by 87% and 55%, respectively, when planted before the nematode-susceptible crops.

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

Therapeutic Protein in Plants that Prevents Cattle Disease. ARS scientists at Beltsville, Maryland, developed a modified plant virus which produces 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 biologically active protein. ARS scientists in the Plant Sciences Institute, Beltsville, Maryland, made use of a fruitful collaboration with animal scientists at the Beltsville Agricultural Research Center to make available an engineered bovine protein and in vivo testing opportunities. An invention report describing the production of a bovine therapeutic protein in plants has been filed with the Office of Technology Transfer, and a determination of patentability is under consideration.

Interactive Web-Based Tools To Estimate Stem Rust Disease Developed. Stem rust is the most important and damaging disease of grasses grown for seed in the Pacific Northwest, requiring over 400,000 pounds of fungicide annually, at an estimated cost of \$10 million and interactive web-based tools to estimate stem rust disease development are needed. Scientists in the Forage Seed and Cereal Research Unit, Corvallis, Oregon, developed a web-based decision making tool that was made available to commercial grass seed growers. The model allowed the prediction of stem rust epidemics and the working model was linked to a website (<http://pnwpest.org/cgi-in/stemrust1.pl>). Delivery of this model allows disease managers to assess the timing and benefits of fungicide sprays and reduce costs through improved efficiency of disease control measures.

Functional Replacement of Wheat Streak Mosaic Virus (WSMV) HC-Pro. The HC-Pro gene of WSMV was systematically replaced with the corresponding gene from closely related strains of WSMV and from distantly related virus species. All HC-Pro replacements were functional in wheat with respect to systemic infection. The experiments demonstrate that HC-Pro genes from dissimilar sources and limited structural similarities nonetheless have conservation of function with respect to pathogenicity in wheat. Alterations in host range and symptom severity for some HC-Pro replacements indicates that HC-Pro may encode determinants of pathogenicity. It is anticipated that the population genetics data base generated for WSMV will facilitate the emerging area of molecular epidemiology, in which the origin and biological properties of pathogens responsible for new (or recurring) epidemics may be accurately and quickly identified based on nucleotide sequence analysis of pathogen populations. Improved understanding of virus replication, movement, host range, and transmission may lead to new ways for controlling the disease and reducing yield losses. Approximately a dozen Material Transfer Agreements have been established with other scientists interested in using the WSMV infectious clone (modified as a gene expression vector), most of whom plan to use the WSMV gene vector as a tool in functional genomics projects for wheat, barley, and other cereal crops.

Transmission of Serious Bacterial Disease of Citrus. The bacterium, *Xylella fastidiosa*, causes serious diseases in many agronomic, horticultural, and landscape ornamental

plants-including Pierce's disease of grape and variegated chlorosis of citrus. If introduced into the United States, the citrus strain would be particularly devastating to citrus production in California and Florida. The pathogen is known to be transmitted by xylem-feeding insects. ARS scientists at Beltsville, Maryland, established that the sweet orange strain of *Xylella fastidiosa* can be transmitted through seed to seedling progeny. This accomplishment is important because this is the first demonstration of seed transmission with this emerging pathogen of global significance.

Component V. Host Plant Resistance to Disease

Identity of Genes Required for Disease Resistance. A virus-induced gene silencing (VIGs) system was demonstrated to work very effectively in barley and wheat and that it can be used as a rapid way to identify genes required for disease resistance. ARS scientists at West Lafayette, Indiana, have identified many genes that may be involved in disease resistance but without a system like VIGs it has been impossible to determine if they are necessary for disease resistance. Proof-of-concept testing demonstrated that these VIGs can silence a chlorophyll synthesis gene, a wheat leaf rust resistance gene, and two additional genes that were postulated to be key components of this resistance pathway. When silenced the resistant plants became either white due to a lack of chlorophyll or susceptible to the wheat leaf rust fungal pathogen. It is now possible to determine the function of genes in hexaploid wheat which was not possible before. This system will be used by wheat and barley scientists world-wide to determine the function of genes.

Mechanism of Disease Resistance Elucidated. Understanding the mechanism of disease resistance is vital for developing integrated management strategies to combat plant diseases. ARS scientists at Albany, California, in collaboration with UC Berkeley scientists characterized a mutation that was identified by a suppressor screen of N gene mediated TMV resistance. The work is important as this mutant appears to mediate resistance signaling for a number of pathogens, including bacterial, fungal and viral pathogens in addition, the results show that R gene-mediated pathways and general resistance pathways intersect or overlap. The results are extremely important as this mutant is in a pivotal position between pathogens and the ability to respond to disease. Research will lead to the development of disease resistance crops. The Bs4 gene, which provides resistance against *Xanthomonas campestris* pv. *vesicatoria* (Xcv), was cloned. The results are important as the Bs4 gene could be used for providing resistance to other tomato varieties. The results are also important for increasing our knowledge of disease resistance. The identification of disease resistance genes increases our ability to improve crops.

Control of Sugarbeet Disease. Fungal pathogens continue to cause significant disease problems in sugarbeet grown in the United States and world-wide. Cercosporin production by *Cercosporin beticola* has long been speculated to be crucial for the infection of sugarbeet by this fungus, yet conclusive data was lacking. In collaborative research with the University of Florida-Lake Alfred, ARS scientists at Fargo, North Dakota, now provide sound scientific data that demonstrates that targeting the

detoxification of cercosporin, or breeding for cercosporin resistance, would be an effective means to limit leaf spot disease in this crop.

Soybean Cyst Nematode Genetic Linkage Map Developed. ARS scientists at Urbana, Illinois, developed a soybean cyst nematode (SCN) genetic linkage map using mating of virulent and avirulent individual males and females. The importance of this linkage map is that it can provide for genomic localization of important genes such as those involved in parasitism. This research identified the location of the SCN parasitism gene HG-CM-1 (Heterodera glycines chorismate mutase 1) and a molecular marker that is associated with virulence (resistance breaking biotypes) on several sources of resistance in soybean to SCN. 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.

Scientists Characterize the Sun1-1 Mutation. Understanding the mechanism of disease resistance is vital for developing integrated management strategies to combat plant diseases. Scientists at the Plant Gene Expression Center, Albany, California, in collaboration with UC Berkeley scientists characterized the sun1-1 mutation that was identified by a suppressor screen of N gene mediated TMV resistance. The work is important, as SUN1 appears to mediate resistance signaling for a number of pathogens, including bacterial, fungal and viral pathogens. In addition, the results show that R gene-mediated pathways and general resistance pathways intersect or overlap. The results are extremely important as SUN1 is in a pivotal position between pathogens and the ability to respond to disease. Research will lead to the development of disease resistance crops.

Shifts in Mosaic Strains Detected. A survey of virus isolates collected from sugarcane exhibiting mosaic symptoms revealed a shift in strain dominance among the naturally infected plants. In contrast to viruses collected from diseased plants between 1990 and 1995 in which more than 90 percent were SrMV strain H and the remainder strains I and M, approximately 68, 10, and 2 percent of the viruses collected between 2001 and 2004 were strains I, H, and M, respectively; 13 percent was an unknown strain of SrMV; and 8 percent appeared to be strains of a virus other than SrMV or sugarcane mosaic virus that causes mosaic in other locations. Among the 693 plants with mosaic symptoms, 18 were found to contain two virus strains, and two plants were infected with three virus strains. These results demonstrate the ability of the different strains of the SrMV to become predominant, for new strains to emerge, or for a new virus to infect Louisiana sugarcane; and suggest that a mixture of strains be used to screen new germplasm for mosaic resistance.

Resistance to Soybean Rust. Soybean rust was recently reported widespread in several southern states of the United States and now represents a significant threat to the Nation's soybean industry. Determining sources of resistance to rust within soybean genotypes is a key priority so that resistance can be deployed in U.S. cultivars, and damage limited as rust spreads within the United States. ARS scientists in Frederick, Maryland, evaluated nearly 20,000 soybean accessions from the USDA soybean germplasm collection at

Urbana, Illinois, in preliminary screens in the BSL-3 Plant Pathogen Containment Facility at Fort Detrick for resistance to soybean rust. Approximately 300-400 lines showed some resistance and will be tested further with individual rust isolates. Identifying these sources of rust resistance is a key step leading to the development of rust-resistant commercial soybean cultivars that can be planted by growers and reduce potential losses.