National Program 303 • PLANT DISEASES

FY 2015 Annual Report

National Program 303, Plant Diseases, focuses on developing effective disease management strategies that are environmentally friendly, safe for consumers, and compatible with sustainable and profitable crop production. This USDA-Agricultural Research Service (ARS) National Program is conducted in cooperation with related research in other public and private institutions. In particular, NP 303 projects are coordinated with those in National Program 301 (Plant Genetic Resources, Genomics, and Genetic Improvement) 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, while safeguarding the environment. To this end, projects in this national program aim to reduce the impact of diseases on yields, product quality or shelf-life, aesthetic or nutritional value, and potential contamination of food and feed with toxins.

Management of plant diseases is essential for providing an adequate and consistent supply of food, feed, fiber, and aesthetic plants, and has long been a high priority for 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.

NP 303 consists of 59 projects located in 19 different states and the District of Columbia. Most of the more than 110 scientists working within this national program are specialists in plant pathology and/or nematology. Significant contributions to NP 303 also come through multidisciplinary teams that include geneticists, agronomists, botanists, horticulturists, physiologists, soil scientists, entomologists, chemists, and microbiologists.

NP 303 is comprised of the following four components:

- *Diagnostics, Etiology, and Systematics*
- *Biology and Epidemiology of Plant Disease*
- *Plant Health Management*
- *Alternatives to Preplant Methyl Bromide Soil Fumigation*

Together, these components include research to understand and control plant diseases and to develop and transfer strategies for disease management and control that enhance agricultural production and value. During fiscal year 2015, this program produced many important discoveries and advances. Some of these are described below, grouped by program component.

Component 1 – Diagnostics, Etiology, and Systematics

Assays for toxin production in bacterial pathogens. *Rathayibacter toxicus* is a USDA-APHIS select agent because infections produce a toxin in forage grasses that is lethal to livestock, resulting in $40 million of damage yearly to Australia. Despite the potential threat to U.S. agriculture and food supplies, the mechanism of toxin production for *R. toxicus* was not known. Scientists at the USDA-ARS Foreign Disease-Weed Science Research Unit in Fort Detrick, Maryland, and the Emerging Pests and Pathogens Research Unit in Ithaca, New York, completely sequenced the genome of three *R. toxicus* strains plus an associated bacteriophage, and determined that the genes responsible for toxin production are part of a transposable element housed in the *R. toxicus* DNA. From the genome information, assays capable of identifying any toxin-producing Rathayibacter species were developed. The assays will be useful for protecting against the introduction of the select agent plant pathogen to the United States, as well as for study of mechanisms by which toxin production is initiated.

Characterization of the novel pathogen, *Fusarium secorum*, causing disease in sugarbeet. A new pathogen was isolated from sugarbeet plants from the Red River Valley. This pathogen appeared to be a Fusarium sp., but showed distinct symptoms and caused disease earlier in the growing season compared to previously known Fusarium species. ARS scientists in Fargo, North Dakota, in collaboration with researchers at North Dakota State University and the Institute of Sugar Beet Research in Göttingen, Germany, carried out molecular and morphological analyses to formally name this pathogen as the new species *Fusarium secorum* and the disease that it causes as Fusarium yellowing decline. Since Fusarium yellowing decline has been increasing in incidence in the Northern Plains sugarbeet growing region, the identification and characterization of the causal agent is a significant first step for the development of disease management strategies.

Multiplex PCR assay to detect and differentiate the Select Agent strains of *Ralstonia solanacearum.* The bacterium, *R. solanacearum* race 3 biovar 2 causes destructive brown rot of potato and is capable of surviving and infecting at low temperatures. Current regulations for Select Agents by Federal and State agencies restrict movement of all strains of *R. solanacearum* unless further testing can exclude them from the race 3 biovar 2 group. ARS researchers in Beltsville, Maryland, developed a multiplex PCR assay to simultaneously (1) detect *R. solanacearum* at the species complex level, (2) specifically identify whether the strain is race 3 biovar 2, and (3) exclude false negatives due to unsuccessful DNA extraction or PCR inhibition. This new, rapid, accurate, and reliable detection assay can help government officials and regulatory agencies make timely and appropriate recommendations to exclude this Select Agent from the United States.

Raspberry bushy dwarf virus characterized from Ecuador. ARS researchers in Corvallis, Oregon, in collaboration with colleagues in Ecuador, identified a new strain of Raspberry bushy dwarf virus (RBDV) in commercial blackberry (*Rubus glaucus*) in Azuay, province of Ecuador, and named the virus RBDV-Ec-Az. The virus contains an extra RNA compared to all other RBDV isolates that have been sequenced. This extra RNA contains a complete copy of RNA2, plus an extra copy of the coat protein in reverse order at the 5' end of the RNA which is a unique duplication among plant viruses, and its significance is still being evaluated. The extra RNA was present in approximately 40 percent of the isolates from Azuay province, but it was not detected in Tungurahua province, which is the main blackberry production region for Ecuador. The ends of RNA2 of this strain of RBDV were similar to the resistance-breaking strain reported from the United Kingdom, while the portion of RNA2 that codes for proteins was more similar to isolates from Slovenia. This strain of RBDV may be a combination of several different strains of RBDV from widely diverse regions, suggesting the international movement of RBDV in plant material.

Blueberry mosaic associated virus characterized. Blueberry mosaic disease was first described more than 60 years ago and has been reported from most blueberry growing regions in the world. ARS scientists in Corvallis, Oregon, in collaboration with colleagues in Arkansas, Michigan, and in Slovenia have characterized a virus associated with blueberry mosaic disease. To develop reliable diagnostic tests, scientists partially sequenced 59 isolates of Blueberry mosaic associated virus to determine its diversity. The isolates were collected from throughout North America, as well as several from Europe. The virus showed very low genetic diversity, and diagnostic primers were designed based on the 59 sequences. Using primers based on a broad range of virus isolates gives confidence that the diagnostic assay developed will detect all isolates of this virus. The assay has been used for testing of blueberries in the National Clean Plant Network, and it is available for certification and quarantine purposes.

New detection assay for mosaic viruses infecting sugarcane using loop-mediated isothermal amplification (LAMP) assay. Accurate and timely detection of pathogenic viruses that cause mosaic in sugarcane plants is important for managing the disease in moderately susceptible varieties and preventing the spread of the pathogens. ARS researchers at the Sugarcane Research Unit in Houma, Louisiana, developed loop-mediated isothermal amplification (LAMP) assays for the specific detection of the two destructive viruses, Sugarcane mosaic virus (SCMV) and Sorghum mosaic virus (SrMV). The advantages of the LAMP assay compared to the conventional polymerase chain reaction (PCR) assay are that the reaction can be completed in a single tube incubated at a constant temperature, the reaction time is approximately one hour compared to two hours, and a positive reaction results in a visual color change of the reaction mixture, which can be easily determined. The LAMP assay, therefore, does not require costly instruments required for conventional PCR or an electrophoresis system to detect the products of positive reactions. Therefore, the LAMP assay is useful for rapid detection and diagnosis, can be efficiently performed with limited resources, and can be adapted for field application.

Component 2 – Biology and Epidemiology of Plant Disease

Fungicides effective against chrysanthemum white rust. *Puccinia horiana* is a foliar plant pathogen that causes the disease chrysanthemum white rust (CWR) and is a significant quarantine pathogen in the United States, and it is eradicated when it is found. Appearance of symptoms typically occurs during the fall just as growers are preparing to ship their chrysanthemum crops. To expand the list of fungicide options for growers to use to control CWR, researchers with ARS in Fort Detrick, Maryland, and with Rutgers University assayed *in vitro* germination of *P. horiana* basidiospores with varying concentrations of 14 fungicides. The strobilurin class of fungicides and fungicide combinations of trifloxystrobin + triadimefon and boscalid + pyraclostrobin were most effective in inhibiting spore germination. This information will be useful to government, academic, and private sector researchers for targeting fungicides to control CWR and for reducing the opportunity for resistance to develop in the pathogen population.

Detection of new races of stem rust in Africa. Wheat stem rust is a fungal disease of wheat that can significantly impact crop yield. A strain of the wheat stem rust fungus known as Ug99 threatens global wheat production due to its ability to infect nearly all wheat varieties. Inadvertent introduction of this pathogen to the United States would severely impact our wheat production. A stem rust race belonging to the Ug99 race group, TTKSK, was detected by ARS scientists in St. Paul, Minnesota, for the first time in Egypt, and two new races (TTKTK and TTKTT) in this group were identified from samples collected in Kenya. These new races attacked the wheat resistance gene SrTmp, an important stem rust resistance gene carried by several newly released cultivars in eastern Africa. These new virulence races explained continued stem rust epidemics in Kenya on newly released Ug99-resistant cultivars, and pose an unrelenting threat to wheat production. Constant vigilance by ARS scientists is necessary to assure continued development of resistant wheat varieties for growers in the United States.

Mitigation measures defined for the economically limiting boxwood blight pathogen. ARS researchers in Fort Detrick, Maryland, showed that microscopic survival structures (termed microsclerotia) of the boxwood blight pathogen can persist for over 30 months in moist sand. Thus, a method was needed by the nursery industry to mitigate infested potting mix, greenhouse surfaces, and nursery beds. The researchers determined the efficacy of selected chemical sterilants against boxwood blight microsclerotia using a unique assay, and the results were included in recommendations made by the State of Virginia Extension Service. This information is now available to stakeholders in the nursery industry and will play a critical role in reducing the economic impact of boxwood blight.

Targeted management of Hop powdery mildew. Powdery mildew is the most costly disease affecting the U.S. hop industry, with disease related costs exceeding 15 percent of crop value annually. ARS scientists at the Forage Seed and Cereal Research Unit in Corvallis, Oregon, and scientists at Washington State University, identified a specific period of susceptibility in juvenile hop cones, and discovered that the outcome of entire disease management programs largely depends on the efficacy of disease control measures applied during a three-week period in the juvenile stages of cone development. Targeting control measures to this critical period nearly doubles the degree of disease control observed at harvest. Results have been widely distributed to the U.S. hop industry through various avenues and have impacted production practices used by over half of producers. Yield loss from powdery mildew in susceptible varieties has been reduced by 2.6 percent industry-wide, which is conservatively estimated at over $2 million annually.

Prediction model and decision aid for rust in perennial ryegrass seed crops. Rust, caused by *Puccinia graminis* subsp. *graminicola*, is one of the destructive diseases of perennial ryegrass seed crops. ARS researchers in Corvallis, Oregon, in collaboration with Oregon State University, developed and evaluated a ryegrass stem rust model (STEMRUST_G). In large plot experiments in commercial ryegrass seed production fields, the decision aid produced disease management outcomes (management costs and seed yield) as good as, or better than, growers' standard practice. The model is currently available to growers through Oregon State University Integrated Pest Management Center as an on-line decision aid for rust management.

Release of three new soybean germplasm lines with moderate resistance to charcoal rot. Charcoal rot reduces soybean yields in stress environments and is ranked in the top four among economically important diseases in the United States. Annual yield losses range from $500,000 to $1 billion. ARS researchers at the Jackson, Tennessee worksite and in Stoneville, Mississippi, developed and released three soybean lines with moderate resistance to charcoal rot. These lines are useful source materials for soybean breeders to incorporate charcoal rot resistance into high-yielding soybean cultivars/lines. Seed has been made available to university and private sector soybean breeders who are actively using these new sources to develop commercial varieties.

Component 3 – Plant Health Management

Canine disease detection for citrus greening. Fifteen years of canine disease detection research by ARS scientists in Fort Pierce, Florida, as part of a HLB Multiagency Coordination Group, has culminated in training 10 dogs to detect Huanglongbing and three dogs to detect citrus canker all at greater than 99.97 percent reliability. Dogs will be deployed over the next two years to various affected states, and commercialization plans are in progress with dog training companies. This is currently the only method to quickly detect citrus greening in trees prior to symptom development. Early detection will aid in management of the disease.

Developing and utilizing a novel screening method to discover and release a soybean variety with resistance to two different nematodes and multiple fungal pathogens. In the United States, annual soybean yield losses from the soybean cyst nematode (SCN) alone exceed $650 million, and resistant cultivars have stabilized yield losses at current levels. But, over time, nematode populations have adapted and overcome resistant cultivars. Current methods available to identify the resistance reaction of soybeans are labor intensive and time consuming. Therefore, ARS researchers at the Jackson, Tennessee, worksite, in collaboration with researchers from University of Tennessee and from University of Georgia, developed a marker-assisted strategy and released a new soybean line, JTN-5203, with broad resistance to two damaging nematode species, while at the same time providing resistance to the predominant fungal diseases: frogeye leafspot, stem canker, sudden death syndrome, and reniform nematode. These scientists also developed a more efficient method for high-throughput selection of SCN resistance. The DNA markers developed differentiate the two most commonly used sources of resistance: Peking and PI 88788. The method is highly useful to soybean breeders and is being widely adopted. Despite its resistance to multiple pathogens, which normally reduce yield potential, yields of JTN-5203 are identical to the check variety 'Ozark.' Growers in non-transgenic production systems will greatly benefit from this conventional soybean. The variety can also serve as an excellent source material in crosses for developing more desirable cultivars. JTN-5203 has been selected to serve as the "standard check" for USDA-Southern Soybean Uniform Tests.

Identification of a wheat protein involved in sensitivity to the mycotoxin, deoxynivalenol. Fusarium head blight (FHB) is a devastating disease of wheat and barley. Losses from FHB in the United States are estimated to have exceeded $3 billion since 1990. This disease not only greatly lowers grain yield and quality, but it causes the grain to become contaminated with a dangerous mycotoxin, deoxynivalenol (DON). DON is harmful to wheat and barley, as well as to humans and animals that consume grain products. DON acts as a disease virulence factor for FHB, and tolerance of DON enhances wheat resistance to *Fusarium* head blight (FHB) disease. ARS scientists in West Lafayette, Indiana, together with researchers at the University College of Dublin in Ireland identified two wheat genes that encode transporter proteins that remove DON from plant cells. A gene-silencing system that can stop the expression of chosen wheat genes was used to turn off these transporters, resulting in wheat that became much more sensitive to DON. Inversely, overexpressing the transporter genes could reduce DON and result in higher resistance to FHB.

Development and release of a mustard green cultivar with resistance to a new bacterial disease, Brassica leaf blight. Beginning in 2005, annual losses to Brassica leafy greens (which include mustard and turnip greens, collards, and kale) production due to a bacterial leaf blight have increased at an alarming rate, ranging from 20-80 percent. Some of the largest growers in the United States, located in South Carolina, have sustained significant losses to this disease, forcing them to find new, non-infested land in other southern states in order to meet demand for their products. These new fields have subsequently also become infested by the pathogen. A new *Brassica juncea* mustard green cultivar, 'Carolina Broadleaf,' developed at the ARS Vegetable Research Laboratory in Charleston, South Carolina, was released and has proven to be exceptionally resistant in small- and large-scale production trials conducted over the past several years. Carolina Broadleaf has been determined to be acceptable for both fresh-cut and canning/processing markets. This is the only known mustard green cultivar, and the only Brassica leafy green cultivar, with resistance to this disease. ARS scientists are currently working with several seed companies regarding production of commercial quantities of seed to meet grower demand.

Identification of wild walnut species/genotypes resistant to crown gall. Crown gall (CG) is a disease that debilitates young trees and facilitates development of other root-destructive diseases in walnuts. ARS scientists in Davis, California, identified open pollinated (OP) seedlings from selected walnut and wingnut (a walnut relative) mother trees which exhibit elevated levels of CG resistance over multiple dormancy periods. CG resistant hybrid progeny from directed crosses were shown to segregate for CG resistance. CG resistant trees, both OP genotypes and interspecific hybrids, have been cloned through *in vitro* propagation and are currently being examined in field trials. These CG resistant genotypes are essential for development of CG resistant rootstocks for the walnut industry.

Isolation and characterization of a tomato gene for resistance to three races of Fusarium wilt. The fungus Fusarium oxysporum l*ycopersici* (Fol) causes devastating wilt diseases of many important crop plants. The tomato genes I-3 and I-7 confer resistance *to* Fol race 3, the cause of Fusarium wilt of tomato. Both genes were bred into cultivated tomato, *Solanum lycopersicum*, from the wild relative *Solanum pennellii*. Gene I-3 was identified previously and encodes an S-receptor-like kinase, but little was known about I-7. ARS scientists at the Plant Gene Expression Center in Albany, California, and collaborators at The Australian National University in Acton, Australia, isolated the I-7 gene, showed that it encodes a leucine-rich-repeat receptor-like protein (LRR-RLP), and found that I-7 also confers resistance to Fol races 1 and 2. The distinct Fol resistance characteristics conferred by I-7, and associated molecular markers make it a useful additional tool in the tomato breeder's toolbox.

Wheat genes for resistance to stripe rust identified and mapped. Growing resistant cultivars is the most effective, economical, easy-to-use, and environmentally friendly approach for control of stripe rust. New genes for effective resistance are needed to diversify the resistance sources used in breeding programs to improve the durability of resistance in commercial cultivars. In 2015, ARS scientists from Pullman, Washington, completed mapping studies of two genes for effective all-stage resistance in spring wheat germplasm 'PI 182126,' two genes for non-race specific high-temperature adult-plant resistance and one gene for all-stage resistance in spring wheat germplasm 'PI 195097,' and one gene for all-stage resistance in differential winter wheat cultivar 'Tyee.' These genes and their molecular markers are useful for breeding wheat cultivars with resistance to stripe rust and also to better characterize races of the pathogen.

Targeted disruption of plant receptor to generate novel nematode resistance in crop plants. Methods for effective control of agriculturally significant nematode pests, including potato cyst nematodes (PCN) and soybean cyst nematodes (SCN), are very limited and often rely on highly toxic chemicals such as methyl bromide. Using resistant plant varieties is the most effective and sustainable means for nematode control. ARS scientists in Ithaca, New York, in collaboration with scientists at the University of Missouri have identified receptors in potato and soybean that bind nematode-secreted proteins and allow the nematodes to parasitize the plants. Using a gene silencing approach, transgenic soybean and potato plants with suppressed expression of the receptor genes were generated and found to have increased resistance to PCN or SCN infection. This technology (U.S. Provisional Patent Application numbers: 61/371,619, 61/507,478; International Patent Application numbers: PCT/US2011/43882, PCT/US12/46631) is generating new nematode resistant potato and soybean varieties that can be used in nematode control and eradication programs in the United States.

Component 4 – Alternatives to Preplant Methyl Bromide Soil Fumigation

Anaerobic soil disinfestation (ASD) as an alternative to methyl bromide fumigation. Anaerobic soil disinfestation, a biological method of soil fumigation, combined with soil solarization was identified as an effective soilborne disease management approach. In the absence of methyl bromide soil fumigation for the production of vegetables in raised planting beds, ASD and soil solarization was shown to be a viable alternative to methyl bromide. It was also demonstrated that a single crop of in-ground cut flowers can be produced using ASD; however, multi-plantings are not recommended following a single treatment with ASD, as was possible with methyl bromide. The combination of ASD and soil solarization can be substituted for methyl bromide for a single crop, providing growers a viable alternative to methyl bromide.

Development of a grower-oriented forecasting system for risk management of Sclerotinia in canola. At present, more than ninety percent of the U.S. canola crop is grown in North Dakota each year, and losses from Sclerotinia represent a major challenge to profitability. ARS leads the National Sclerotinia Initiative, and collaborators from North Dakota State University have developed a grower-oriented forecasting system, consisting of a general risk map and a risk calculator, designed to assist producers with managing Sclerotinia - http://www.ag.ndsu.edu/sclerotinia/. The general risk map utilizes weather information to estimate risk of disease development throughout the canola-growing areas of the state. This map is updated twice weekly and posted on-line, beginning in mid to late June and continuing throughout the canola flowering period each season. The 2015 risk calculator combines information on cultural practices, the field-specific past history of Sclerotinia, and current weather information - retrieved from the nearest weather station - to estimate the risk of disease development. This on-line tool is currently being used by producers throughout North Dakota for the selection and timing of cultural and chemical interventions against Sclerotinia.