United States Department of Agriculture
Agricultural Research Service

National Program 303 • PLANT DISEASES

FY 2018 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 ARS' 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 identify plant pathogens and reduce crop losses caused by plant diseases, while safeguarding the environment. To this end, projects in this national program aim to limit the spread of plant diseases, which thereby reduces the impact of diseases on yields, product quality or shelf-life, aesthetic or nutritional value, and potential toxin contamination of food and feed.

Management of plant diseases is essential for providing an adequate, safe 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 without increasing 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 50 projects located in 17 different states and the District of Columbia. Most of the more than 120 scientists working within this national program are specialists in plant pathology and/or molecular biology. 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 encompasses the following three components:

- *Component 1 – Etiology, Identification, Genomics and Systematics*
- *Component 2 – Biology, Ecology, and Genetics of Plant Pathogens and Plant-Associated Microbes*
- *Component 3 – Plant Health Management*

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 2018, this program produced many important discoveries and advances. Some of these are described below, grouped by program component.

Component 1 – Etiology, Identification, Genomics and Systematics

Diagnostics for Macrophomina root and crown rot. With the banning of methyl bromide for soil fumigation, *Macrophomina phaseolina* is an emerging pathogen in California strawberry production. ARS researchers in Salinas, California, and collaborators at University of California, developed two different types of molecular diagnostic tests that are specific for the Macrophomina types capable of infecting strawberry. The tests measure the amounts of the fungus in the soil as well as provide a means for rapid detection directly in the field. Isolates of the fungus that are highly infectious on strawberry are, with a few exceptions, genetically identical. These assays provide researchers with the tools they need to identify the pathogen and provide growers with the ability to determine risk prior to planting.

American potato disease outbreak: causative agent identified in New York State commercial potato production. Starting in 2014, there has been a destructive and costly outbreak of blackleg disease in U.S. and Canadian commercial potato production. There are several bacterial pathogens known to cause this disease depending upon the location. It is important to properly identify the pathogen causing the disease so that proper control measures can be applied. ARS Scientists in Ithaca, New York, tested potato plants provided by potato growers located in New York to determine the cause of blackleg disease on these plants. They found that approximately half of the disease-causing bacteria belonged to the genus *Pectobacterium*, which is a well-known persistent low-level problem. The other disease-causing bacteria were *Dickeya dianthicola*, a newly identified and unusually virulent species. This information is important as it will be used by scientists, breeders, and extension agents since knowing the cause of the potato blackleg disease outbreak is the first step in devising solutions such as improved diagnostic tools and improved methods for sanitation to curtail the spread of the disease.

Improved detection method that differentiates two citrus pathogens. Spiroplasma citri causes citrus stubborn disease (CSD), which has symptoms that are easily mistaken for Huanglongbing (HLB), caused by *Candidatus* Liberibacter asiaticus (CLas). CSD is fairly widespread in California but is a manageable disease of citrus; whereas, HLB is a devastating citrus disease subject to quarantine and immediate removal of infected trees. ARS researchers in Parlier, California, developed a sensitive procedure to identify and quantitate S. citri and CLas from citrus and vector tissue using a technique called droplet digital PCR (ddPCR), which was more sensitive and reliable than the standard molecular technique when there is little pathogen amounts present. The ddPCR test provides a robust method to test symptomatic and non-symptomatic citrus samples for CSD and HLB in a single test, saving time and money, and may be used by regulatory agencies as an improved testing procedure to differentiate between deadly HLB and the more benign CSD.

Tracking down the origins of wheat disease leads to new ideas on prevention. Wheat and barley crops are vulnerable to a disease called Fusarium Head Blight (FHB) which can impact farmers by reducing yield and requiring costly fungicide applications. ARS scientists in St. Paul, Minnesota, tracked down the origin of the fungus that causes this costly disease. Remarkably, many native grasses found along highways and hedgerows can harbor the fungus, and unlike the case of crop plants, these native grasses allow the fungus to grow without causing symptoms of FHB. While the non-symptomatic plants potentially could be a reservoir of the harmful fungus, an interesting prospect is that the plants themselves may contain genes for tolerance to the fungus and resistance to the disease it causes.

Genome comparison of three fungal isolates that cause disease in wheat. Septoria nodorum blotch (SNB) is a major problem for wheat growers in the United States, causing substantial yield and quality losses due to infection by the pathogen *Parastagonospora nodorum*. ARS researchers in Fargo, North Dakota, obtained and compared genome sequences for three *Parastagonospora nodorum* strains, including a strain collected from wild grass, a strain collected from durum wheat, and a strain collected from bread wheat. These strains were sequenced, and evaluation of the three genomes showed that one of the chromosomes was completely absent in the strain collected from wild grasses. This suggests the potential for dispensable chromosomes involved in disease and provides the necessary foundation for investigation of how wheat pathogens are causing disease. This knowledge is critical to intelligent disease control and wheat breeding.

Devised and improved sensitivity of technology for phytoplasma detection. Phytoplasma, a class of pathogenic bacteria, causes serious crop diseases, but is often found in low concentration in plants making it difficult to detect. In collaboration with the University of Florida, ARS scientists in Beltsville, Maryland, adapted a new technology known as digital PCR (dPCR), devised a protocol for its application as a superior approach for sensitive detection of palm infecting phytoplasmas, compared to other commonly used PCR platforms, and described the first use of the technology in phytoplasma detection. This work will facilitate the application of quarantine measures designed to prevent the spread of palm and other phytoplasmas across national borders. Such progress will aid the causes of food security, sustainable agricultural production, and stewardship of natural ecosystems. This report will interest diagnostics laboratories, research scientists, and farmers, as well as APHIS and international quarantine agencies.

Diagnostic test developed for alfalfa quarantine pathogen. The presence of the quarantine pathogen, *Verticillium alfalfae*, in alfalfa products (hay or seed) prevents the export or import into states and countries where the pathogen has not been previously found, resulting in trade restrictions and significant economic losses. Previous detection tests did not distinguish the specific *V. alfalfae* from other *Verticillium* species, or the required specialized experience, expensive equipment, and significant time to use. ARS researchers in Corvallis, Oregon, developed a rapid, easy-to-use, inexpensive test that can be used by growers, diagnostic labs, and regulatory/inspection agencies to differentiate and detect *V. alfalfa* from other related species in exported or imported alfalfa products.

Rapid assessment of fungicide resistance in powdery mildew affecting grape and wine production. Powdery mildew is a fungal disease common to vineyards, especially in the Pacific Northwest and California. Growers have a low tolerance for infected fruit because of the impact on wine quality, and the disease can devastate grape production if uncontrolled. In 2015, vineyards in western regions reported crop losses due to powdery mildew disease which was resistant to strobilurin fungicides, which are commonly used for chemical control. But there was no way to know if a vineyard was infected with resistant grape powdery mildew until the chemical controls failed, and the crop was damaged. Therefore, ARS scientists in Corvallis, Oregon, in collaboration with Washington State University and Michigan State University, identified the genetic mutation that is associated with strobilurin resistance and developed rapid sampling and detection methods so that growers can rapidly test for the presence of strobilurin resistant grape powdery midlew in their fields and adjust fungicide selection for chemical control. In 2017 and 2018, over 3,000 samples were assessed from California, Washington, and Oregon, with greater than 85% of samples having strobilurin resistant grape powdery mildew. Growers participating in the survey using the rapid detection system substantially reduced crop losses, and researchers are tracking resistance to strobilurin fungicides to develop better control strategies.

Component 2 – Biology, Ecology, and Genetics of Plant Pathogens and Plant-Associated Microbes

Mating disruption of glassy-winged sharpshooter by playback of vibrational signals through vineyard trellis. Glassy-winged sharpshooter (GWSS) is an important vector of the bacterium *Xylella fastidiosa*, the causal agent of Pierce's disease of grapevine. GWSS insects communicate by exchanging mating calls that are transmitted through host plants as vibrational signals. ARS scientists in Parlier, California, showed that interference with GWSS communication by playback of disruptive, vibrational signals through vineyard trellis systems significantly reduced mating of GWSS on grapevines. Although further studies are needed prior to commercial adoption, data from this study support application of vibrational mating disruption as a novel method to control GWSS populations.

Glyphosate (Roundup) has only subtle and minor effects on soil microbes. The herbicide glyphosate is the most widely used herbicide in the United States and is a key tool in the direct-seed no-till system that reduces soil erosion and fossil fuel inputs. However, growers in the Pacific Northwest have been concerned about non-target effects on soil microbes such as bacteria and fungi, which perform beneficial functions. ARS scientists in Pullman, Washington, using next-generation sequencing, compared microbial communities in treatments with and without glyphosate, that were taken from fields with a long history and no history of glyphosate use. They showed that the effects of glyphosate were very minor and that location and cropping system had much larger effects on fungal and bacterial communities. In fact, more soil bacterial and fungal communities were increased with glyphosate use due to the nutrients provided by dying roots. This is valuable information for farmers who are concerned about glyphosate and want to continue to use this important tool, as previously there was little scientific literature on this topic.

Documentation of 500 new nematode specimens completed. Plant-parasitic nematodes are microscopic roundworms that attack plant roots and cause an estimated $10 billion of crop loss each year in the United States and $100 billion globally. One problem with reducing these crop losses is that nematode species are notoriously difficult to identify in part due to a lack of reference materials. ARS scientists in Beltsville, Maryland, completed the documentation for nearly 500 additional nematode species for the USDA Nematode Collection over the last 20 years. This information is significant because it provides knowledge of the numbers and kinds of nematodes that are known to exist and for which specimens can be obtained from the Nematode Collection. The specimens are used by scientists, extension agents, growers, and quarantine officials to anticipate and accurately identify these nematode species for research and pest control.

Spinach pathogen in leaves detected prior to symptom development. Downy mildew disease of spinach, caused by the plant pathogenic microorganism *Peronospora effusa*, is a major disease constraint on spinach in the United States and worldwide. The period between plant infection and symptom development is known as the latent period. ARS researchers in Salinas, California, led the effort to detect DNA of the pathogen in the latent period in spinach leaves. Early detection of the pathogen in the leaves in the field will help to more effectively target fungicide applications prior to symptom development or to harvest organic crops earlier, and thereby help to prevent downy mildew epidemics.

Radical new understanding of the basic biology of *Moniliophthora roreri*, causal agent of frosty pod rot of cacao. Frosty pod rot of cacao, caused by the fungus *Moniliophthora roreri*, is the greatest threat to cacao production globally, should it escape the Americas. In the initial disease phase, the pathogen avoids detection (compatible phase) as it grows throughout cacao fruit tissues before switching to a destructive phase and rapidly destroys the fruits. For many years, it was thought that *M. roreri* was haploid in the compatible phase, having one nucleus per cell, before converting to a dikaryotic stage, carrying two nuclei per cell. Having a dikaryotic phase is critical to sexual reproduction in a fungus like *M. roreri* and plays a large role in the generation of genetic diversity in the fungus. ARS scientists in Beltsville, Maryland, used nuclear staining to examine the nuclear content of *M. roreri* throughout its life cycle and discovered that the fungus never forms true dikaryotic cells. This information redefines the lifecycle of *M. roreri*, supporting the idea *M. roreri* lacks the ability to undergo sexual reproduction. The implication of this finding means that frosty pod resistant cacao trees should remain stable in the field with a limited risk of the pathogen overcoming the resistance through genetic recombination. These findings will impact all cacao improvement programs, globally, and as new resistant materials are identified, breeders will be able to supply farmers with cacao trees that will remain productive for extended periods of time, despite the presence of this disease.

Virus associated with select agent *Ralstonia solanacearum* affects its virulence. *Ralstonia solanacearum* is a bacterial pathogen that causes millions of dollars of crop losses in a wide range of plant species worldwide. One strain in particular, the r3b2 subgroup, is such a threat to U.S. agriculture that it has been designated a select agent in the United States and is a quarantine pathogen in Europe and Canada. ARS scientists in Beltsville, Maryland, discovered a virus associated with the bacterium that offers the bacterium a competitive advantage. The virus appears to play a role in regulating the virulence of its carrier bacterial strain and in helping the bacterium to persist in the environment, which in turn prolongs the symbiotic relationship between the virus and the bacterium. This study helps define the relationship between the virus and the bacterium so that effective controls for this important plant pathogen can be developed.

Component 3 – Plant Health Management

Release of disease resistant germplasm from wild sunflowers. Sclerotinia basal stalk rot (BSR) and downy mildew are two fungal diseases that are major yield limiting factors in global sunflower production. The use of resistant hybrids, where available, is the most efficient and environmentally friendly means of managing these diseases. ARS scientists in Fargo, North Dakota, transferred resistance to BSR from three species of wild annual sunflowers into cultivated sunflower, resulting in the release of seven sunflower germplasm lines. All lines except one also contain resistance to downy mildew derived from one of the parents. These lines represent the first oilseed sunflowers with resistance to Sclerotinia BSR and downy mildew together and are being used across the United States and internationally to breed for resistance to multiple diseases that reduce seed quality and severely impact yield.

Characterization and selection of a new highly-effective oat crown rust resistance gene from wild oat, *Avena strigosa*, into cultivated oat. Oat crown rust (*Puccinia coronata* f. sp. *avenae*) is a major disease that yearly damages global oat production. ARS scientists in St. Paul, Minnesota, identified a new, highly-effective resistance gene to oat crown rust from wild oat, *Avena strigosa*, and introduced it into cultivated oat through a technique called marker assisted selection. This gene confers broad resistance (*i.e.*, field resistance to a wide diversity of the pathogen population) to this devastating disease of oat, making it highly valuable to scientists around the globe.

PhylloLux technology for crop protection. New approaches are needed for controlling strawberry diseases as currently used strategies relying mainly on fungicides have significant limitations due to reduction in their effectiveness caused by development of resistance in plant pathogens, possible restrictions on their use, and increasing public demand for produce free of pesticide residues. ARS researchers in Kearneysville, West Virginia, developed PhylloLux technology, a plant disease management system that combines Ultraviolet C (UV-C) irradiation followed by a specific dark period with the application of biocontrol agents. The PhylloLux system can also be used to control mites – the major arthropod pest in strawberry production. Microbiome analysis revealed no major shift in the composition of the microflora of fruits and leaves that would indicate increase of food-borne pathogens after antagonist treatment. The potential of this technology goes well beyond its application to strawberries and may include applications in production of other fruit and vegetable crops as well as ornamental plants and nursery stocks.

Germplasm screening for resistance to wheat blast accelerated wheat variety release. Wheat blast, caused by the Triticum pathotype of the fungus *Magnaporthe oryzae*, is a devastating disease of wheat. Although the pathogen spread locally in South America for nearly 30 years, the disease was not reported elsewhere until 2016 when an outbreak occurred in Bangladesh. The outbreak resulted in significant yield loss in the affected area and threatened the release of a biofortified wheat variety, Bari Gom-33, developed in Bangladesh. In collaboration with the Mexico-based International Maize and Wheat Improvement Center (CIMMYT) under a cooperative agreement with Kansas State University, the variety was tested by ARS scientists in Ft. Detrick, Maryland, for resistance to wheat blast. The testing revealed good levels of resistance and enabled the accelerated release of the variety Bari Gom-33 for farmers in Bangladesh to grow.

Six disease-resistant sugarcane cultivars for Florida growers to mitigate the impact of disease. The biggest challenges facing sugarcane growers in Florida are orange rust and brown rust disease, which cause considerable yield losses and increase costs due to repeated fungicide applications. Orange rust was first identified in Florida in 2007, so many of the most widely-planted, high-yielding sugarcane cultivars are susceptible. Therefore, ARS researchers in Canal Point, Florida, in collaboration with the University of Florida and Florida Sugar Cane League, identified and developed new sugarcane cultivars with resistance to rust diseases. In 2018, six cultivars were released by the Florida Sugarcane Variety Committee, three for production in muck soils and three for sand soils. In addition to orange and brown rust resistance, these new cultivars have high yields for increased profitability. Florida produces twenty percent of the sugar consumed in the United States, and cultivars CP 11-1314, CP 11-1956, and CP 11-2248 for muck soils and CP 10-1620, CP 10-1716, and CP 10-2195 for sand soils are expected to increase sustainable and profitable production.

Joint effort resulted in the release of two new nematode resistant potato cultivars. Potato cyst nematodes (PCN) are serious pests for U.S. potato production which is valued at $4 billion. Host resistance is the most effective and environmentally-sound method for PCN control. ARS researchers in Ithaca, New York, in collaboration with scientists at Cornell University have developed and released two new golden nematode resistant potato cultivars, ‘Algonquin’ and ‘Upstate Abundance’, both of which are early season table-stock cultivars. These cultivars can serve as an effective tool for PCN control and eradication in the United States and can be utilized by potato breeders as parent material in crosses to develop more resistant cultivars.

Fruit of watermelon from resistant germplasm lines are resistant to fruit rot at all stages of development. Watermelon is an important crop grown throughout the United States. In recent years, a disease called Phytophthora fruit rot is re-emerging as a serious problem in many watermelon production areas in the United States. Weather conditions for fruit rot development are always prevalent in the southeastern United States, and hence the pathogen can infect the fruits at any stage. ARS researchers in Charleston, South Carolina, identified, developed, and released Phytophthora fruit rot resistant germplasm lines for use by seed companies and University plant breeders. They also showed that watermelon fruit from susceptible commercial varieties were highly susceptible at all fruit stages while USDA-developed resistant germplasm lines were resistant at all fruit ages. These findings suggest that disease management actions may need to begin as early as fruit set for commercial cultivars that are susceptible and currently being cultivated. The fruit rot resistant watermelon germplasm will be useful for public and private plant breeders for incorporating fruit rot resistance in watermelon cultivars.

Improved management of cucurbit downy mildew. Downy mildew can cause devastating losses to cucurbit and hops production. Development of an effective pathogen monitoring program would enable a more efficient spray program to prevent disease. ARS researchers in Salinas, California, and collaborators at North Carolina State University, developed a multiplexed molecular marker-based system for detecting and measuring the amounts of three downy mildew pathogens on cucurbits and hops. These markers have been shared with collaborators and will provide a means for rapid identification of the pathogen and improved management of fungicide spray programs for enhanced disease control.

Improved aflatoxin reductions in the field using non-toxigenic strains applied with a bioplastic. Aflatoxins are a group of very stable, potent carcinogenic mycotoxins produced by the fungal species *Aspergillus*. The most effective method available now to manage this problem in corn is biological control using non-toxigenic *Aspergillus flavus* strains. Planting fully-protected seed can result in an 82.5% reduction in aflatoxin levels. ARS scientists in Stoneville, Mississippi, in collaboration with colleagues at the University of Bologna, Bologna, Italy, optimized the application method using seed treatment techniques of a corn-starch-based formulation, called “bioplastic.” This novel technique reduced the dosage of the applied biocontrol isolates but still maintained effectiveness in reducing aflatoxin contamination in corn. The impact of this study further strengthens the case for using biological control to reduce aflatoxin contamination and will help reduce the preparation costs for use by the public and private sector, including ARS, academia, and industrial grain producers.

Mapping the stripe rust resistance genes in winter wheat variety ‘Skiles’. Stripe rust can cause major losses to wheat producers world-wide. It is best controlled through growing resistant varieties, and genes with tightly linked molecular markers are needed for breeding programs to develop new varieties with durable and high-level resistance. ARS scientists in Pullman, Washington, mapped six genes for the high-level, non-race specific high-temperature, adult-plant (HTAP) resistance in Pacific Northwest winter wheat variety ‘Skiles’ and determined their different effect levels and interactions to growth stage and temperature. This work demonstrates the effective approach of combining several genes to achieve an adequate level of durable type resistance. Markers were developed for the resistance genes using the new Komptitive allele-specific PCR (KASP) technique which were validated by testing breeding lines. The genes and markers identified in this study can be used in breeding programs for efficiently developing new stripe rust resistant varieties.

Cloning of a wheat stem rust resistance gene from diploid wheat. Wheat stem rust is a devastating disease that is threatening global wheat production. The emergence of new virulent races of this pathogen in Africa, including the Ug99 race group, has prompted global efforts to find effective resistance genes. In collaboration with researchers at University of California-Davis, ARS scientists in St. Paul, Minnesota, identified the stem rust resistance gene Sr21 that is effective against the Ug99 race group. A diagnostic marker was developed to accelerate its deployment in wheat breeding programs, and it demonstrated that the introduction of two Sr21 copies in transgenic wheat results in high levels of resistance. An unusual characteristic of Sr21 is its increased resistance to stem rust at high temperatures. Identification of temperature-sensitive Sr21 will be useful for controlling the stem rust pathogen in a changing global environment.

Effective soilborne disease and weed management in forest nurseries. Seventy percent of the $350 million Pacific Northwest forest nursery industry still uses methyl bromide (MB) soil fumigation to manage soilborne diseases and weeds in tree seedling nursery beds. ARS researchers in Corvallis, Oregeon, in collaboration with forest nursery industry partners, conducted field trials to evaluate other fumigants besides MB for disease and weed control. Totally impermeable films (TIF) were tested to reduce fumigant emissions from the soil, and fumigants were applied below label rates to reduce chemical inputs and meet buffer zone requirements for nurseries located next to suburban housing developments. Two alternative fumigants were identified (metam sodium + chloropicrin and 1,3-dichloropropene + chloropicrin) that were effective at controlling soilborne diseases and weeds when applied below label rates under TIF. These results provide effective alternative fumigants for soilborne disease and weed control, while reducing both chemical inputs, costs, and emissions. As a result of this research, forest nursery industry partners have adopted reduced-rate MB as a standard operational treatment, and most nurseries are now using TIF to reduce emissions.