

**United States Department of Agriculture  
Agricultural Research Service**

**National Program 303 • PLANT DISEASES**

**FY 2019 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 125 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.

Many of the NP 303 projects include significant domestic and international collaborations including government, industry and academia. These collaborations provide opportunities to leverage funding and scientific expertise for USDA-ARS research and accelerate dissemination of ARS research results, thus enhancing the impact of ARS research programs. During FY 2019, NP 303 scientists participated in research collaborations with scientists from Argentina, Australia, Austria, Azerbaijan, Belgium, Bhutan, Bolivia, Brazil, Cambodia, Canada, Chile, China, Colombia, Costa Rica, Czech Republic, Denmark, Ecuador, Egypt, Ethiopia, Finland, France, Georgia, Germany, Ghana, Greece, India, Indonesia, Ireland, Israel, Italy, Japan, Jordan, Kazakhstan, Kenya, Lithuania, Malaysia, Mexico, Morocco, Nepal, Netherlands, New Zealand, Pakistan, Palestinian Territories, Peru,

Philippines, Poland, Russia, Rwanda, Saudi Arabia, Scotland, Slovenia, South Africa, South Korea, Spain, Sri Lanka, Sweden, Switzerland, Taiwan, Tanzania, Thailand, Trinidad and Tobago, Tunisia, Turkey, United Kingdom, and Uruguay.

The quality and impact of NP 303 research was evidenced during FY 2019 by the following:

- ✓ 305 refereed journal articles published,
- ✓ 5 new invention disclosures submitted,
- ✓ 1 new patent application filed, and
- ✓ 2 new patents issued.

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 2019, this program produced many important discoveries and advances. Select accomplishments are described below, grouped by program component.

### **Component 1 – Etiology, Identification, Genomics and Systematics**

**Plant pathogen toxins lethal to livestock.** *Rathayibacter agropyri* is a plant pathogen closely related to *R. toxicus*, an APHIS-listed select agent found in Australia that produces a toxin in forage grasses that is lethal to livestock. *R. agropyri* is endemic to the U.S. Pacific Northwest. ARS researchers in Fort Detrick, Maryland, have sequenced the entire DNA genome of several strains of *R. agropyri*. The genomes of some, but not all, *R. agropyri* strains encode all genes needed to synthesize a tunicamycin-like toxin. The *R. agropyri* toxin genes are related to toxin biosynthetic genes from *R. toxicus* and *Streptomyces chartreusis*, two bacteria that are known to make tunicamycin-like toxins. Although it is not known whether or under what conditions *R. agropyri* might produce a tunicamycin-like toxin, the discovery of a complete and likely functional toxin biosynthetic cluster (TGC) in at least some *R. agropyri* strains makes it possible that toxin production could occur. Identification of a new TGC highlights the conserved nature of tunicamycin-like antibiotics within the *Rathayibacter* genus and emphasizes how little is known about many of them.

**Huanglongbing strains in southern California have different origins.** ‘Candidatus Liberibacter asiaticus’ (CLas) is a bacterium associated with huanglongbing (HLB) that was first detected in Florida in 2005 and in California in 2012. HLB has devastated citrus throughout Florida and has now been detected in more than 1,494 infected citrus trees in urban southern California. The only way to control HLB is to remove infected trees. Repeated insecticide application to kill the Asian citrus psyllid (ACP), the insect that serves as a vector for HLB, is neither economically, environmentally, nor biologically sustainable. ARS researchers in Parlier, California, along with scientists from the California Department of Food and Agriculture, the Animal and Plant Health Inspection Service, and South China Agricultural University, examined genomic diversity among 10 CLas strains from southern California by next-generation sequencing and detected prophages in all CLas strains. The California strains formed four

prophage groups associated with different collection sites and were more closely related to strains in Asia rather than Florida, which indicated that the Clas pathway of entry was from independent introductions at multiple times. This information is important to growers in formulating HLB management strategies for different pathogen strains and for regulatory agencies in prioritizing and optimizing pathogen interdiction surveys.

**A new sensitive method to detect low titer samples of ‘*Candidatus Liberibacter asiaticus*’ (Clas).**

Clas, which is vectored by the Asian citrus psyllid, is the bacterium associated with the devastating citrus disease huanglongbing (HLB). Eradication of Clas inoculum sources is the first line of defense against the spread of HLB but erratic distribution and low titer of the pathogen in citrus limits efficacy of diagnosis using real-time polymerase chain reaction (qPCR) to detect the targeted Clas 16S rRNA gene. ARS researchers in Parlier, California, along with a scientist in the Central California Tristeza Eradication Agency, developed duplex real-time PCR and droplet digital PCR (ddPCR) tests to definitively detect low-titer Clas samples using two different genetic targets (16S and RNR) in the Clas chromosome. Sensitivity of the duplex assay was shown to detect down to two DNA copies per typical PCR reaction volume. Using low-titer (marginally positive) samples of Clas in leaf tissue and ACP, detection of Clas was greater with the RNR than the 16S gene target. Therefore, the duplex ddPCR described above can be used to confirm questionable results based on the regulatory standard 16S target.

**Increased diagnostic efficiency for citrus pathogens.** Citrus fruits are collectively one of the most important U.S. horticultural crops, with a farmgate value of more than \$3 billion in 2018. Citrus species are threatened by many virulent diseases that threaten the U.S. crop and genetic resource collections. But accurate molecular diagnostic techniques—which can provide early warning of disease occurrence—involve very costly DNA and RNA extraction kits. ARS scientists in Riverside, California, and Beltsville, Maryland, devised and evaluated two less expensive methods to extract nucleic acids from citrus plants. One of these methods provided a 90 percent cost savings without losing diagnostic accuracy, and the second method yielded a 65 percent cost savings. These methods will provide significant cost savings to diagnostic laboratories once they are validated and approved by USDA-APHIS.

**Distribution of cereal cyst nematode in eastern Washington.** Cereal cyst nematodes account for about \$51 million in annual losses in dryland wheat in the Pacific Northwest. However, little was known about where these nematodes occur. ARS scientists in Pullman, Washington, surveyed eastern Washington over a 10-year period and developed a molecular method to distinguish *Heterodera avenae* from *H. filipjevi*, two species that are morphologically difficult to distinguish. They found that *H. avenae* is widely distributed in the highest precipitation zone where crops are grown every year, but not as much in a dry wheat-fallow area. *H. filipjevi* is confined to southern Whitman County. This information is crucial for growers to know their risks, and information about the species is critical for breeders to develop cereals with resistance to the nematodes, because different resistance genes are effective against different nematode species.

**A newly identified third virus associated with lettuce dieback disease.** Lettuce dieback causes necrosis, stunting, and death of lettuce in the western United States where approximately 80 percent of U.S. lettuce is grown. Infection often results with a complete loss of crop. Two related and highly stable soilborne *Tombusvirus* species are known to cause the disease. In recent years, disease symptoms have been increasingly observed in plants that are not infected by either of these known viruses, suggesting an additional virus may be involved. RNA sequencing and small RNA analysis of

previously collected diseased samples from throughout the Salinas Valley region by an ARS researcher in Salinas, California, and University of California Cooperative Extension staff led to identification and partial sequence of an unknown virus that is distantly related to a recently characterized member of the *Phenuiviridae* family of viruses. Archived samples and additional field samples were subsequently evaluated for presence of the new virus, tentatively named lettuce dieback associated virus (LdaV), and show a high correlation between presence of the virus and disease. Characterization of the relationship between LdaV and disease development will clarify its importance in disease development and epidemiological importance for lettuce production, leading to improved knowledge of factors contributing to disease and benefitting the lettuce industry.

**New diagnostic tests for two emerging pathogens of strawberry.** After the loss of methyl bromide for soil fumigation, two emerging pathogens, *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *fragariae*, have become more problematic for strawberry growers in California. An ARS researcher in Salinas, California, and University of California collaborators developed two different types of molecular diagnostic tests specific for the *Macrophomina* and *Fusarium* types capable of infecting strawberry; the tests measure the amounts of each fungus in soil and provide a means for rapid detection directly in the field. Isolates of the fungus that are highly infectious on strawberry are, with few exceptions, genetically identical. These assays provide diagnosticians and researchers with the tools they need to identify the pathogen and growers with the ability to determine risk prior to planting.

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

**Genetic characterization of a threatening cotton pathogen on the move.** A highly virulent cotton wilt fungal pathogen known as *Fusarium oxysporum* f. sp. *vasinfectum* race 4 (Fov4) was found in cotton in West Texas in 2017; until then, the disease had been confined to California since 2001. Although most of the genetic types of Fov that are found in U.S. agricultural fields require the presence of nematodes to cause disease, Fov4 causes severe disease in the absence of nematodes and represents a major threat to cotton production. ARS scientists in College Station, Texas, working on Fov4 collections from various locations, identified four distinct genetic groups within Fov4 and, using modern molecular techniques, clearly defined their relationships with each other. Specific detection methods incorporating a molecular technique known as polymerase chain reaction were developed for identifying Fov4 and distinguishing the four genetic subtypes. These methods will be extremely valuable in precisely identifying the specific Fov4 subtype in a given field or in harvested cotton seed. This accomplishment represents a significant advancement in Fov4 research that will help minimize the further spread of this devastating disease and facilitate the work of cotton breeders in developing Fov4-resistant varieties.

**Microscopy studies determine basil downy mildew rapidly germinates on leaves.** Downy mildew caused by *Peronospora belbahrii* (an oomycete) is a devastating disease of sweet basil (*Ocimum basilicum*) production worldwide. Traditional methods for reducing plant disease, such as spraying fungicides, requires using a variety of chemicals to limit the possibility of the pathogen developing resistance. Organic basil growers have very limited means to control this disease. Documenting how the pathogen infects basil plants and how quickly it produces spores would help scientists

know when in the pathogen's lifecycle it is most vulnerable to control measures. ARS scientists in Peoria, Illinois, used a scanning electron microscope to determine that spores of the pathogen on basil leaves germinated in 3 days and the mildew directly penetrated leaves soon after. After 7 more days, spore-producing reproductive structures of the pathogen formed on both the bottom and top of young leaves, which enhances dispersal to other plants. These results contribute to more knowledge of the infection process and indicate that downy mildew control products should be present on all leaf surfaces or introduced into the plant systemically, which should lead to the design of better crop protection products for the control of downy mildew.

**Population genomics of a worldwide collection of wheat leaf rust reveal long-distance migration.** ARS scientists in St. Paul, Minnesota, used a collection of 565 *Puccinia triticina* isolates, the causal pathogen of wheat leaf rust, from 11 worldwide wheat growing regions to genotype them using the sequence-based genotyping technique. The regional populations were analyzed for nucleotide variation and genetic relationships using 6,745 single nucleotide polymorphisms (SNPs). Populations from Russia and Central Asia, North and South America, Europe, and Pakistan, and New Zealand and South Africa were highly related for SNP genotypes. Isolates from durum wheat found around the world were highly different for SNP genotype compared with all isolates that originated from common wheat. Specific isolates from collected from durum wheat in Ethiopia were unrelated to any previous isolates from durum or common wheat. Recent isolates from Europe, North America, South America, Ethiopia, and Pakistan with virulence to one leaf resistance gene (Lr17a) and avirulent to another (Lr2a) were highly related for SNP genotype, which indicated migration of these races and SNP genotypes across global regions. A molecular clock phylogenetic approach, based on SNP variation between isolate genotype groups and mutation rate, determined that isolates from Ethiopia with virulence only to durum wheat are the oldest form of *P. triticina* on wheat, followed by isolates found worldwide with virulence to durum wheat, and then isolates with virulence to common wheat. This finding, published in the journal *Phytopathology*, should help develop a more effective plan to combat this disease in wheat.

**Developing the life cycle model for the northern root-knot nematode in Washington vineyards allows better management strategies.** Wine grape growers in Washington do not have adequate methods to manage plant parasitic nematodes. Over 2 years at multiple sites, ARS scientists in Corvallis, Oregon, and researchers from Washington State University characterized the development dynamics of the northern root-knot nematode, the most common nematode parasite in Washington vineyards. This nematode produced only one generation per year. By gaining insight into the biology of this pest in production vineyards, it is now possible to improve management strategies, such as the application of pesticides. For example, it is now known that the susceptible stage of this nematode is most abundant in soil in the spring and fall. These findings may be used by wine grape growers to better time control strategies to reduce the impact of this damaging pest.

**Development of a cryopreservation method for nonsporulating fungi.** Long-term preservation of experimental fungi without loss of their genetic and pathological properties is critical for plant pathology investigations. Existing methods can be cumbersome, hazardous, expensive, and often not suitable for long-term storage of fungi that do not easily produce spores under laboratory conditions. ARS scientists in Beltsville, Maryland, developed a method for preservation of

nonspore-forming fungi in commercially available porous beads (Microbank™) under low-temperature (–80°C) conditions without loss of morphological, pathological, and genetically identifiable characteristics. Research findings demonstrated the utility of low-temperature preservation in Microbank beads as a convenient method for long-term storage of a wide group of fungi for plant pathology investigations. This information will be useful to scientists investigating the biology and management of sterile pathogenic fungi.

### **Component 3 – Plant Health Management**

**Improved walnut rootstocks with resistance to key plant pathogens.** The United States is second in walnut production in the world, 99 percent of which is grown in California. Unfortunately, current rootstock choices for walnut growers offer little protection against diseases such as crown gall, lesion nematodes, and *Phytophthora* crown and root rot. An exciting rootstock improvement program was created through a partnership between public breeding programs and private industry, including the California Walnut Board. New rootstocks from that partnership have recently been confirmed through long-term orchard survival studies. ARS scientists in Davis, California, in collaboration with University of California–Davis researchers and commercial walnut growers, confirmed the superior resistance to *Phytophthora cinnamomi*, the most aggressive cause of crown and root rot on California walnuts, in field trials using a walnut rootstock hybrid, RX1, which was previously selected for resistance to *Phytophthora* in greenhouse trials. In an orchard infested with *P. cinnamomi*, more than 30 percent of trees on standard seedling rootstock died within 2 years after planting, whereas no trees on RX1 rootstock died after 8 years of the ongoing trial. These findings validate superior resistance of the RX1 rootstock under real-world commercial conditions.

**First soybean germplasm for broad resistance to soybean cyst nematode.** Soybean cyst nematode populations are diverse, a characteristic that helps this destructive pest overcome plant resistance. Currently three sources of soybean exist that have genetic nematode resistance and this can be used in commercial soybean varieties, but they all can be defeated by an existing race of the nematode pathogen. ARS scientists in Jackson, Mississippi, discovered resistance in a plant found in the USDA National Plant Germplasm System that offers broad resistance to multiple races of the pathogen. They developed three new germplasm lines from crossing the soybean germplasm line JTN-5503, which contained original sources of resistance via the cultivar ‘Hartwig’, to the newly discovered resistance source PI 567516C, now providing very broad resistance to multiple nematode populations in the field. Genetically, the resistance in the new source is different from the three traditionally used sources of resistance that are currently available. PI 567516C provides broader resistance to multiple nematode populations and is even more stable in combination with at least one of the previously existing resistance sources. Thus, the shifts in nematode races will be minimized, resulting in reduced yield losses for sustainable soybean production. All three new germplasm releases are excellent parental materials and are being used to broaden the genetic base of soybeans while breeding new cultivars for nematode resistance without yield reductions.

**Stripe rust resistance genes in winter wheat variety ‘Eltan’ mapped.** Stripe rust is a major disease of wheat and is best controlled by growing resistant varieties. ‘Eltan’ is a wheat variety

that is widely grown in the Pacific Northwest, but its genes for stripe rust resistance were unknown. ARS scientists in Pullman, Washington, mapped five genes for resistance to stripe rust in ‘Eltan’. Two major genes and a minor gene conferred race-specific all-stage resistance and two minor genes conferred non-race-specific high-temperature adult-plant (HTAP) resistance. They determined that the reduction of stripe rust resistance in ‘Eltan’ in recent years was due to the current predominant rust races overcoming the race-specific all-stage resistance genes and that the level of HTAP resistance was low. Their results, published in the journal *Phytopathology*, highlight the importance of combining high-level HTAP resistance and effective all-stage resistance for developing wheat varieties with high-level, durable resistance to stripe rust. The markers identified for HTAP resistance genes in ‘Eltan’ are useful for combining with other effective resistance genes for developing new wheat varieties.

**Efficacy of selected biopesticides in reducing the severity of soybean rust has potential to reduce antimicrobial resistance.** Soybean rust is a fungal disease that has produced yield losses of up to 60 percent in the southern United States. The intensive use of synthetic fungicides for controlling soybean rust has accelerated the emergence of fungal populations with reduced sensitivity to synthetic fungicides. To provide alternatives to synthetic fungicides, ARS scientists in Urbana, Illinois, cooperated with a University of Illinois researcher to determine whether application of biopesticides would reduce sporulation of the soybean rust fungus. One biopesticide made from the bacterium *Bacillus subtilis* caused particularly high reductions in sporulation of the soybean rust fungus. The identification of biopesticides that effectively reduce soybean rust may help develop valuable alternatives or complements to synthetic fungicides in integrated pest management programs for soybean rust control. This information is important to soybean growers, extension agents, and others interested in the use of nonsynthetic fungicides to control soybean rust.

**Identification, characterization, and introgression of new genetic sources with resistance to peanut pathogens.** The genetics of resistance to aflatoxin accumulation in peanut seeds is complex. The lack of an effective phenotyping method has hindered the development of resistant cultivars or alternative methods of control. ARS researchers in Dawson, Georgia, have developed a new method to screen for aflatoxin resistance in the laboratory that to date has allowed the identification of aflatoxin resistance in wild peanut germplasm. The study uncovered gene expression differences in the peanut/fungus interaction between susceptible germplasm and germplasm with aflatoxin resistance. Results from this study provided unique insights into defense strategies used by peanut seeds against *Aspergillus* infection and aflatoxin accumulation. In addition, ARS researchers in Dawson, in collaboration with other peanut researchers, published the first two reports on peanut smut genetic resistance. The studies identified smut resistance in both wild *Arachis* species and peanut landraces, and further demonstrated its introgression into elite peanut cultivars. These findings are expected to have a significant impact on peanut breeding programs worldwide.

**Developing tools to combat antimicrobial resistance in a postharvest fungus of apples.** Apples are one of the most consumed fruit in the United States and are stored for 6 to 12 months. During storage, the fruit becomes increasingly susceptible to rot caused by the blue mold fungus, which reduces quality, contributes to waste, and produces the mycotoxin patulin. A new postharvest fungicide for apple fruit was released for use in 2015. ARS researchers in Beltsville, Maryland, in

collaboration with Cornell University, determined the sensitivity to patulin of a globally diverse *Penicillium* spp. population that cause blue mold, developed a discriminatory dose to monitor antimicrobial resistance, and tested the patulin formulation against different fungicide-resistant blue mold isolates. A discriminatory dose for difenoconazole is being used by extension professionals to detect fungicide-resistant strains to maintain efficacy of current postharvest chemicals in the mid-Atlantic and Pacific Northwest regions.

**Field evaluation of integrated management for mitigating huanglongbing (HLB) in Florida.** ARS and University of Florida researchers in Fort Pierce, Florida, implemented an integrated HLB management strategy that includes chemotherapy, thermotherapy, and additional nutrition treatment in field trials over 3 years. To assess the complex interactions, they used several methods to evaluate the effectiveness of integrated management, including the slopes of the cycle threshold ( $C_t$ ) increase used for detection of 'Candidatus Liberibacter asiaticus' (Clas) bacterium, the pathogenic index and the decline index from  $C_t$  value and tree scores, and the antimicrobial efficacies from pathogenic and decline indices. This comprehensive analysis showed that most of the tested chemicals were effective to some degree in killing or suppressing Clas. Trunk-injected penicillin was the most effective chemical treatment in all groves, followed by oxytetracycline, and silver nitrate delivered as foliar sprays. Although steam heat treatment and additional nutrition did not eliminate or suppress Clas over the long term, these treatments did positively affect tree growth and recovery in the short term. Overall, the results provide new insights into HLB control methods and strategies for integrated management for HLB epidemic plantations.