

**United States Department of Agriculture  
Agricultural Research Service  
National Program 303 • PLANT DISEASES  
FY 2017 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 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 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 54 projects located in 19 different states and the District of Columbia. Most of the more than 140 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 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 2017, 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**

**New disease-causing viruses affecting petunias and black-eyed Susans discovered.** Ornamental crops are a major contributor to the U.S. agricultural production sector. Like food and fiber crops, there are many viruses that can infect ornamentals and cause yield and quality losses. This is especially true when

the primary value of the plant is in the floral aesthetics. ARS scientists in Beltsville, Maryland, discovered a new virus infecting petunias and a new virus infecting black-eyed Susans (*Rudbeckia hirta*). In both cases, the viruses were transmitted to other hosts and verified as the causal agents of the symptoms. Researchers also determined the complete sequence of the genomes of both viruses and gave them tentative names. Petunia chlorotic mottle virus (PCMoV) is a rod-shaped RNA virus, and Rudbeckia flower distortion virus (RuFDV) is a spherical DNA virus. Furthermore, these researchers also discovered a known mite-transmitted RNA virus infecting roses in Minnesota. These findings are useful to the ornamental industry, regulatory agencies, and other scientists; they can help determine whether additional regulations are needed to prevent the spread of the viruses, as well as provide the basic information needed to develop detection methods that help support virus management strategies.

**Detection of plant quarantine pathogen *Ralstonia solanacearum* r3b2 with portable POKKIT™ and BLItz® systems.** *Ralstonia solanacearum* (Rs) is a bacterial species 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, and regulations require that all strains of Rs be designated as select agents until proven to be non-r3b2. ARS and APHIS scientists in Beltsville, Maryland, collaborated with scientists at Rutgers University to develop two portable platforms for Rs r3b2 detection - the POKKIT™ and the BLItz® - that significantly increase the sensitivity, speed, specificity, accuracy and portability of diagnostic assays for Rs in order to prevent the Rs r3b2 strain from entering the U.S., and also to avoid unnecessary exclusion of non-r3b2 Rs strains. The palm-sized POKKIT™ can be used with previously published primers to detect as few as 10 bacteria in 32 minutes, including heat-inactivated samples and in plant tissue, and the BLItz® instrument has comparable speed and sensitivity to the commercial ImmunoStrip® with the advantage of higher r3b2 specificity. These two independent, portable systems can facilitate Rs r3b2 detection at the ports of entry and in field settings.

**Computational tools for characterizing microbiomes developed.** Community-level sequence data, the type generated by an increasing number of metabarcoding studies, is often graphed as stacked bar charts or pie graphs that use color to represent taxa, which are limited in conveying the hierarchical structure of taxonomic classifications. As an alternative, scientists in Corvallis, Oregon, developed metacoder, an R package for easily parsing, manipulating, and graphing publication-ready plots of hierarchical data. Metacoder includes a dynamic and flexible function that can parse most text-based formats that contain taxonomic classifications, taxon names, taxon identifiers, or sequence identifiers. Metacoder can then subset, sample, and order these parsed data using a set of intuitive functions that take into account the hierarchical nature of the data. Finally, an extremely flexible plotting function enables quantitative representation of up to 4 arbitrary statistics simultaneously in a tree format by mapping statistics to the color and size of tree nodes and edges. The package complements currently available tools for community analysis and provides an open source with an extensive online user manual for scientists in academia, government, and the private sectors who are interested in conducting population genomic analysis and manipulating these data.

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

**Grain contaminant vomitoxin made in subcellular "toxin factories."** Harmful byproducts of fungi, called mycotoxins, are important risks to human and livestock health. Little is known about how fungal mycotoxins are produced and how the toxin is delivered to contaminated cereal grain. ARS researchers in St. Paul, Minnesota, have discovered that the toxic fungal product vomitoxin, which can contaminate

wheat and barley grain, is produced in specialized portions of fungal cells called toxisomes. Changes in the fungus containing toxisomes convert cells into "toxin factories" capable of delivering vomitoxin to wheat and barley grains. This information will be important for developing novel strategies for preventing contamination of grain with vomitoxin.

**The genome of *Spiroplasma citri*, the causative agent of Citrus stubborn disease, is sequenced.**

*Spiroplasma citri* causes serious disease in citrus and other economically important plants. ARS scientists in Beltsville, Maryland, achieved the first completely assembled genome of *Spiroplasma citri*, thereby providing valuable information needed for improved *Spiroplasma citri* detection and for understanding how spiroplasmas spread and adapt to their hosts. The decoded *Spiroplasma citri* genome sequence will enable crop pathologists to design and develop new genome sequence-based therapies for the field.

**Molecular genetic characterization of a phytoplasma associated with grapevine yellows disease in Peru.** One of the most destructive diseases in cultivated grapevine plants are grapevine yellows (GY). Yellow diseases around the globe exhibit very similar symptoms, but are attributed to infection by different species of microscopic organisms called phytoplasmas. In the fall of 2015, grapevine plants exhibiting symptoms indicative of a possible yellowing disease were observed in a vineyard located in northwestern Peru. Using DNA fingerprinting technology, ARS scientists from Beltsville, Maryland, with researchers in Peru jointly determined that the disease was caused by a phytoplasma closely related to the reference strain of '*Candidatus Phytoplasma brasiliense*,' a species that was previously not found in Peru and never implicated in a GY disease anywhere in the world. The research team also identified molecular markers for specific detection of the Peru GY phytoplasma. The information is important to the viticulture industry and extension personnel for GY disease diagnosis and management, and is critical to regulatory agencies for preventing exotic pathogens from being introduced into the United States.

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

**Cloning of a durum wheat stem rust resistance gene.** Wheat provides a substantial proportion of the calories and proteins consumed by the human population, but further increases in wheat production are necessary to feed a growing human population. Reducing yield losses caused by pathogens can contribute to these increases. ARS scientists in St. Paul, Minnesota, report the identification and development of perfect markers for Sr13, a gene from durum wheat that confers resistance to the new virulent races of the stem rust pathogen that appeared in Africa at the beginning of this century including Ug99. Two phenotypic alleles, Sr13a and Sr13b, were identified based on their effectiveness to control stem rust pathogen races. The perfect markers developed for Sr13 and knowledge of allelic differences will allow U.S. durum wheat breeders to more effectively develop Ug99 resistant cultivars. In addition, Sr13 can be a useful component of transgenic cassettes that include multiple resistance genes.

**Genetic mapping of the location imparting resistance to root knot nematode in cotton.** The root-knot nematode is widely found throughout the Cotton Belt, and crop rotation with non-host crops can provide short-term nematode suppression. Effective crop rotation usually is a challenge for growers, and host plant resistance is preferred. One of the two known genetic locations imparting resistance to root knot nematode in cotton is on chromosome 14. But this genetic location was only sparsely mapped, and genetic markers were not linked closely enough to the gene location for effective selection. Therefore, ARS researchers in Tifton, Georgia, collaborated with University of Georgia scientists to create new molecular markers that are more closely linked to the gene location, and demonstrated that the resistance gene(s) are confined in a much smaller segment of DNA than previously identified.

Additionally, the researchers used the cotton genome of *Gossypium raimondii*, a precursor of modern cotton, to identify 20 genes in the newly delineated region that could be involved in nematode resistance. The improved markers allow better pin-pointing of the exact resistance gene location for breeding programs, and are being used to develop resistant germplasm.

**Discovery of a unique new source for broad resistance to soybean cyst nematodes.** Soybean Cyst nematodes are wormlike, microscopic organisms that grow and reproduce on the soybean plant roots, reducing seed yields significantly. Worldwide, soybean cyst nematodes are the most destructive pests of cultivated soybean, with annual yield losses in the United States estimated to be nearly \$1 billion. Crop losses are primarily reduced by the use of resistant cultivars for sustainable production. But nematode populations are genetically highly variable, and over time they will change and develop the ability to reproduce on currently grown resistant cultivars. Therefore, it is important to use diverse sources of resistance for broad and more durable resistance. Soybean PI 567516C, a recent introduction in the National Plant Germplasm System from China, was found to be resistant to widely variable nematode populations. ARS researchers in Jackson, Tennessee, crossed elite parental line USDA JTN-5503 with PI 567516C and developed three soybean cyst nematode resistant soybean lines with broad cyst nematode resistance. These new lines are excellent parental materials, and breeders are using them to breed more new cultivars that can produce sustainable yields in the presence of diverse nematode populations.

**Developed new wheat germplasm lines with resistance to stripe rust.** Growing resistant varieties is the most effective, economical, easy-to-use, and environmentally friendly approach to control stripe rust. Germplasm with well-characterized, effective resistance genes is essential and more efficient for developing new resistant varieties. ARS scientists in Pullman, Washington, recently released 29 new wheat germplasm lines (PI 679598 – PI 679626), of which 15 have a single different resistance gene and 14 each have a combination of two linked resistance genes. These lines also have improved agronomic traits compared to the original donors through multi-year selections in the fields. Use of these new germplasm lines will diversify stripe rust resistance genes used in breeding programs, especially the lines with two linked genes that increase the possibility of combining the two different genes into new wheat varieties with high-level, durable or long-lasting resistance.

**A new adult plant resistance gene to crown rust in oat.** A single effective adult plant resistance gene in oat with the associated KASP/SNP markers has been released to various oat breeding programs from across the United States for incorporation into adapted germplasm. The markers have proven effective in the breeding program for selection of the gene in their cultivar development. This new gene and associated markers are critical in developing long-term solutions to the persistent oat crown rust problem in the United States. ARS scientists in St. Paul, Minnesota, observed that over the past two years of release, no crown rust pustules have been identified on lines carrying this gene. Additional genes are being identified for pyramiding and development of an effective package against this highly variable pathogen.

**Biological antifungal alternative to chemical control of potato dry rot disease.** Fusarium dry rot caused by the fungus *Fusarium sambucinum* during storage and transit causes greater losses of both seed and commercial potatoes than any other postharvest disease (up to 25 percent, with as many as 60 percent of the potatoes infected). Chemical control by thiabendazole, currently the only chemical registered for use on food-grade potatoes, is no longer effective, since approximately 80 percent of the dry rot fungi have developed resistance to this chemical. As a biological alternative, novel strains of microorganisms that control potato dry rot disease were discovered by ARS scientists in Peoria, Illinois, as a byproduct of their work with bioenergy feedstocks. The top performing microorganisms imparted excellent protection

to potato dry rot disease. This new anti-fungal control provides a non-chemical, biologically-based alternative to less effective synthetic chemicals. Commercial development of these antifungal microorganisms would also add value as a co-product of the renewable, lignocellulosic-bioconversion industry with the effect of boosting economic viability.

**Identification of a new fungicide for controlling chickpea seed rot.** Chickpea seed rot is caused by metalaxyl-resistant *Pythium*, and *Pythium* seed rot of chickpea has been effectively controlled for more than three decades by applying the fungicide metalaxyl onto seeds before planting. But, over the past three years seed rot caused by certain strains of *Pythium* that are resistant to metalaxyl, has emerged as a threat to chickpea production in the United States. Therefore ARS researchers in Pullman, Washington, conducted both laboratory experiments and field evaluations that clearly demonstrated that the fungicide ethaboxam is effective as a seed treatment in protecting chickpea seeds from seed rot caused by metalaxyl-resistant *Pythium*. As a result of this work, the use of ethaboxam as a seed treatment has been rapidly adopted as a common practice for chickpea production in the Pacific Northwest. This has resolved this emerging disease problem, at least for the present time.

**Optimizing Anaerobic Soil Disinfestation (ASD) as a replacement for methyl bromide.** Given the elimination of methyl bromide from the producer's toolbox, and the increasing regulatory pressure on chemical fumigants, inexpensive and reliable chemical-independent soil fumigation approaches are needed. Anaerobic soil disinfestation (ASD), the elimination of plant pathogens from soil by anaerobic soil microbes and their toxic by-products, is one approach that shows great promise. A key determinant for ASD success, however, is the carbon source used in ASD, which should be easily applied by growers, readily available, inexpensive, and suppressive to a broad range of pathogens and pests. ARS researchers in Davis, California, evaluated 18 different carbon sources derived from agricultural waste and identified the five most effective sources for controlling the bacterial pathogen that causes crown gall disease and other target pathogens. Thus, ASD using agricultural waste provides a reliable and cost effective alternative to conventional soil fumigation.

**Post-harvest storage conditions and plant physiological characteristics that limit progression of potato zebra chip disease.** Zebra chip disease, putatively caused by the bacteria '*Candidatus Liberibacter solanacearum*,' is a major threat to potato production in North America and worldwide. Long-term approaches for production of marketable potatoes rely on understanding the factors that limit zebra chip disease development. ARS researchers in Parlier, California, and collaborators at the University of Idaho, University of California-Riverside, and the Mexican National Institute for Agriculture and Forest Research reported reduced zebra chip symptoms when infected potatoes were kept in storage at 6 or 9 degrees Celsius versus 3 degrees Celsius. Various Mexican-bred potato cultivars that were less susceptible to zebra chip showed fewer changes in the host chemistry when infected, compared to susceptible cultivars, with the end result being potatoes that remained marketable. Results from these studies support the potential use of higher storage temperatures in combination with tolerant cultivars to mitigate the impact of zebra chip disease on potato quality.

**Methodology and software developed to characterize plant canopy 3-D architecture from terrestrial LiDAR data.** A new method was developed and tested by ARS scientists in Corvallis, Oregon, that uses terrestrial Light Detection and Ranging (LiDAR) scanning data to rapidly measure the three-dimensional distribution of leaf orientation and size. The method was validated by comparing LiDAR-measurements to 1) 'synthetic' or computer-generated LiDAR data where the exact orientation and area are known, and 2) direct measurements of leaf parameters in the field using destructive sampling. Overall, agreement between the LiDAR and reference measurements was very good, showing an error of about 15 percent

for the synthetic tests, and 13 percent in the field. This research will significantly enhance understanding of how canopy architecture influences pest and disease development and dispersion, and the ability to model plant growth, photosynthesis, evapotranspiration, and other transport processes that are dependent on understanding leaf orientation in relation to light or wind interception. This method will be of great benefit to grape growers, who can use it to improve their crop growing practices.