

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

National Program 303

PLANT DISEASES

FY 2024 Annual Report

This report synthesizes research and accomplishments from scientists and their support staff working in USDA Agricultural Research Service (ARS) National Program 303 (NP 303), Plant Diseases, from October 1, 2023, through September 30, 2024.

Mission

Develop control strategies to reduce losses caused by plant diseases that are effective and affordable while maintaining environmental quality. The projects in NP 303 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.

Vision

Crops and landscapes protected from plant diseases using scientifically based, environmentally sound, and cost-effective methods.

Performance Measure

Provide scientific information to increase our knowledge of plant genes, genomes, and biological and molecular processes to protect crops and cropping systems from the negative effects of pests and infectious diseases. Develop sustainable control strategies for crop pests and pathogens based on fundamental and applied research that are effective and affordable, while maintaining food safety and environmental quality.

Introduction

NP 303 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. NP 303 projects are coordinated with those in ARS's 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.

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 44 research projects located in 24 different locations. Most of the ~130 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.

The presidentially mandated [National Plant Disease Recovery System \(NPDRS\)](#) ensures the availability of tools, infrastructure, communication networks, and research capacity that is required to mitigate the impact of plant disease outbreaks in the United States and maintain a reasonable level of crop production. ARS allocates money to the NPDRS program to conduct research on new and emerging plant diseases, and in 2024 these funds were distributed across 30 competitive projects to ARS scientists and their collaborators. These projects included diseases that affect alfalfa, avocado, banana, barley, cacao, citrus, common bean, cotton, cucumber, elm, forage grasses, grape, maize, oak, okra, olive, peanut, rice, strawberry, sugarbeet, sugarcane, watermelon, and wheat.

During FY 2024, NP 303 scientists participated in international research collaborations with scientists from Argentina, Australia, Bolivia, Brazil, Canada, Chile, China, Colombia, Costa Rica, Dominica, Egypt, England, Ethiopia, France, Germany, Ghana, Greece, Honduras, India, Israel, Italy, Jamaica, Japan, Jordan, Kenya, Lithuania, Mexico, Morocco, Netherlands, New Zealand, Nigeria, Philippines, Poland, Portugal, Saudi Arabia, Scotland, South Africa, South Korea, Spain, Sri Lanka, St. Lucia, Taiwan, Tanzania, Trinidad and Tobago, Tunisia, Turkey, and the United Kingdom.

Outputs

The quality and effect of NP 303 research was evidenced during FY 2024 by the following:

- 239 refereed journal articles published,
- 4 new invention disclosures (patent only) submitted,
- 22 variety and/or breeding lines released which were evaluated and/or bred for disease/pest resistance, and
- 1 new patent application filed.

Components

NP 303 encompasses the following three research components in fiscal year 2024:

Component 1. Plant Pathogen Resources, Systematics, Genomics, and Diagnostics;

Component 2. Biology, Ecology, and Epidemiology of Plant Pathogens; and

Component 3. Plant Health Management.

Research in Component 1 provides information to understand the cause(s) of diseases and to identify and classify the causal and/or associated microorganisms and their vectors. Through the advancement of molecular tools with traditional techniques, accurate and quick methods are developed that are critical for disease management and for the safe movement of agricultural and horticultural products. Component 2 research builds upon our understanding of how plant pathogens interact with their host and environment. This knowledge may allow us to target weak links in the different phases of the pathogen life cycle. Accomplishments in Component 3 integrate the research from Components 1 and 2

into a systems approach to develop new and effective plant health management strategies. Results provide effective, safe, environmentally-sound, affordable, and sustainable methods that can be implemented by the grower. Together, these components enhance agricultural production and value. During fiscal year 2024, this program produced many important discoveries and advances. Select accomplishments are described below, grouped by program component.

Component 1. Plant Pathogen Resources, Systematics, Genomics, and Diagnostics

Culturing the bacterium that causes citrus greening disease. Citrus greening or Huanglongbing (HLB) is a citrus disease that has caused more than 70 percent yield losses in Florida's commercial citrus groves. To date, the bacterium that is strongly associated with this disease has not been cultured outside of a living host, which complicates efforts to rapidly test control methods. ARS researchers in Fort Pierce, Florida, and University of Florida and Clemson University collaborators developed a semi-selective artificial culture medium and protocol and used the culture in a laboratory setting to successfully infect citrus seedlings and reproduce typical HLB symptoms. Results from the new culturing method, which were published in *Horticulture Research*, enabled the researchers to prove the bacterium was responsible for HLB. This culturing system provides enormous opportunities for accelerating HLB research and better solutions for HLB control.

The krisp python package for developing CRISPR Cas diagnostic assays using whole genome sequences. Pathogens continue to emerge at accelerated rates and the rapid development of novel diagnostic tools is needed to monitor novel pathogen variants or groups. ARS researchers in Corvallis, Oregon, developed the computational tool krisp to identify genetic regions suitable for developing clustered regularly interspaced short palindromic repeats (CRISPR) diagnostics. Krisp scans whole genome sequence data for target and non-target groups to identify diagnostic regions based on DNA or RNA sequences. This computational tool has been validated using genome data for the sudden oak death pathogen. Krisp has been released as an open-source program under a permissive license with all the documentation needed to quickly design CRISPR-Cas diagnostic assays. This technology provides a much-needed novel tool for the biosurveillance of emerging and invasive pathogens.

Genotype-independent wheat transformation system simplifies gene transfer to elite wheat lines. A major impediment for efficiently transferring newly discovered genes directly into elite varieties is the process of plant transformation, in which the gene is introduced to a cell and the cell is grown to regenerate the whole plant. Improving this process makes it possible to introduce or edit genes to improve disease resistance, remove gluteins from seed proteins, or create dwarf phenotypes that were so critical for the green revolution. ARS scientists in West Lafayette, Indiana, and Purdue University collaborators developed a simple and efficient system for wheat transformation that is a gateway for using biotechnology to improve wheat. Unlike all existing methods, the system is genotype independent, so transformation and gene editing can be done directly in current elite wheat varieties or varieties that seed companies are developing for future sale, reducing the years needed to develop new lines that farmers can grow.

Development of a new method for detecting a virus from its insect vector. Impatiens necrotic spot virus (INSV) is transmitted by small insects called thrips. In recent years, INSV has caused hundreds of millions of dollars in losses to the lettuce industry in California's Salinas Valley, where more than 70 percent of all U.S. lettuce is grown. Because thrips are common and widespread, it is difficult to predict when and where INSV outbreaks will occur. ARS scientists in Salinas, California, developed a molecular-based tool to identify thrips populations infected with INSV that is cheaper and more rapid than existing tools. The new tool provides a new surveillance tool for identifying INSV by screening their insect vectors, and advances developing new strategies for predicting virus outbreaks in lettuce crops.

Developing molecular diagnostics for intercepting and identifying an emerging tomato virus. Tomato torrado virus (ToTV) has emerged as a concern for tomato production in many parts of the world, but it has not been identified to date in the United States. The Animal Plant Health Inspection Service (APHIS) needed a reliable method to identify low levels of ToTV on diverse plant materials that may be entering the United States. ARS scientists in Salinas, California, and Corvallis, Oregon, used molecular biology to develop a quantitative diagnostic system for detecting and measuring ToTV from plant samples. The assay includes an internal control for assay validation and is specific for ToTV, showing no cross reactivity with other related viral species. The methods have been provided to APHIS to use in the diagnosis of intercepted plant materials.

Invasive fungi stopped at U.S. borders and characterized. Many plants and plant products entering the United States harbor destructive and invasive fungal pathogens. ARS scientists in Beltsville, Maryland, in collaboration with APHIS scientists in Beltsville, Maryland, isolated and characterized more than 20 distinct species of fungi plant material intercepted at U.S. borders or ports of entry. This enhances the ability of USDA to safeguard U.S. agriculture from foreign pathogens as well as track the movement of pathogens if inadvertently introduced.

Improved detection of citrus greening (Huanglongbing) disease agent through novel sampling methods. Citrus greening disease, which threatens the citrus industry, is caused by the bacterial pathogen '*Candidatus Liberibacter asiaticus*' (Clas). Early detection and prompt response to infections are key factors for eradication of Clas. Sampling citrus trees for detection of Clas is challenging because the pathogen is not evenly distributed in an infected tree. ARS researchers in Parlier, California, in collaboration with the Alliance of Pest Control Districts compared different sampling methods for optimal pathogen detection. Sampling fruit-connected stem tissue from all four sides of each tree was found to give the most consistent detection of Clas compared with other sampling methods currently in use by regulatory agencies. This new development in sampling methods is expected to significantly improve early detection efforts and facilitate Clas eradication in California.

Development of diagnostic markers for an emerging fungal disease of lettuce. Fusarium wilt of lettuce has been an emerging problem in California, Arizona, and Florida production systems and the lack of an effective diagnostic assay has hindered management of the pathogen. ARS researchers in Salinas, California, developed a highly specific and sensitive molecular assay for pathogen detection and quantification of inoculum in the soil. The assay was modified for use with common molecular protocols that can be done directly in the field in as little as 20 minutes. To provide the grower community with resources to manage the disease the assay has been shared with other researchers and commercial diagnostic labs; a manuscript describing the technique is currently under review at a scientific journal.

Development of molecular assays specific to California strains of a disease on strawberry. Fusarium wilt of strawberry in California is typically managed through growing a disease resistant cultivar. Previously, ARS scientists discovered a new strain of the fungus that causes Fusarium wilt on strawberry in California, called *Fusarium oxysporum f. sp. fragariae* race 2 that can cause disease on resistant varieties. This new strain could not be detected with previously published assays for detection of the Fusarium wilt pathogen of strawberry. ARS researchers in Salinas, California, developed new molecular assays to specifically detect this pathogenic strain and have provided them to public and private diagnostics labs across the state. This will enable the rapid detection of this pathogenic strain in new fields so that growers can take steps toward its containment.

Improved methods for monitoring *Phytophthora* populations in irrigation water sources. In California, surface sources of irrigation water such as rivers and canals contribute vitally to crop irrigation and thereby partially offset ground water overdrafts, a chronic problem in the state. However, surface water, unlike groundwater, can contain *Phytophthora* species, some of which are lethal root and crown

pathogens affecting orchards, and growers' use of surface water is therefore tempered by perceived disease risk. Because of technical challenges of characterizing *Phytophthora* populations in irrigation water, little is known about the actual disease risk. ARS scientists in Davis, California, applied high-throughput, long-read DNA sequencing and associated bioinformatic technologies to characterize *Phytophthora* populations at 15 locations in a major California irrigation district. The methods detected 39 species of *Phytophthora*, and compared to previous shorter-read sequencing approaches, provided superior species resolution. The long-read methods are now being applied to examine impacts of surface water use on orchard soil populations of *Phytophthora* species to further examine disease risk.

Optimization of a method for routine detection of an emerging virus in tomato and pepper seeds to reduce dispersion. Tomato brown rugose fruit virus (ToBRFV) is an emerging and rapidly spreading RNA virus that infects tomato and pepper, with tomato as the primary host. The virus is stable and mechanically transmitted, with both standard horticultural practices and contaminated seeds serving as the primary modes of transmission. In collaborative research with scientists from Irapuato, Mexico, ARS researchers in Beltsville, Maryland, examined the relevance of emergent diseases associated with potential seed dispersion of ToBRFV Mexican strains to agronomically important related hosts, focusing on a comparison of molecular biology techniques for rapid and sensitive virus detection in seed lots. An optimized extraction and amplification protocol was developed to achieve optimal sensitivity and accuracy of ToBRFV detection for routine detection in seeds lots and plants, reducing the risk of dispersion. The results of this study, published in the Mexican Journal of Phytopathology, will be of value to the vegetable seed industry, growers, regulatory agencies, and researchers worldwide.

Development of a High-throughput Method for Detecting Guava Root-knot Nematode in Sweet potato. Guava Root-knot nematode (GRKN) is an invasive and quarantined pest that is spreading across the U.S. sweet potato industry. The most popular sweet potato varieties in the United States are highly susceptible to this nematode, and the transport and planting of nematode infected sweet potato 'seed' roots is a major avenue by which GRKN is spread to new fields. To help prevent the further spread of this nematode, ARS researchers in Charleston, South Carolina, developed and successfully tested a high-throughput method for detecting GRKN in commercial sweet potato operations. They provided training and assistance to state extension services to help them implement this new and effective detection method. Compared to traditional nematode survey methods, this new method allows for quicker sampling and more sensitive detection of GRKN in batches of sweet potato roots. This will significantly reduce the labor and technical expertise needed for GRKN sampling and identification and could help slow the spread of GRKN to new sweet potato fields.

Comparing worldwide populations of a pathogen to help breeders. *Macrophomina spp.* cause economically damaging diseases of at least 97 crops, and their impact is expected to increase with abiotic stresses induced by weather effects. ARS researchers in Salinas, California, worked with an international group of scientists to acquire and sequence the whole genomes of more than 400 isolates of *Macrophomina spp.* collected from around the world. These data showed evidence for specialization on soybean and strawberry hosts for a specific species (*M. phaseolina*) that all grouped together. Greenhouse experiments verified that only a single sub-grouping of *M. phaseolina* is highly virulent on strawberry. Evidence for genetic recombination and frequent trafficking of *Macrophomina spp.* between countries was also discovered. These results can help breeders select appropriate isolates to evaluate germplasm for disease resistance. Evidence for genetic recombination indicates a high potential for novel, virulent genotypes to emerge.

Component 2. Biology, Ecology, and Epidemiology of Plant Pathogens

Population analyses and identification of genomic regions associated with aggressiveness of white mold isolates on sunflowers. White mold caused by *Sclerotinia sclerotiorum* impacts numerous important crop species, and some crop resistance has been identified, but responses to different isolates of the pathogen are not always uniform. ARS scientists in Fargo, North Dakota, and colleagues at North Dakota State University and Washington State University evaluated the population characteristics of 219 *S. sclerotiorum* isolates collected from 29 different plant species in 25 U.S. states across the country. These studies revealed differences in isolates from warmer and cooler climates, and genetic mapping identified three genomic regions associated with the vigor of the isolate that infects sunflower. These results are being used to improve disease management strategies and resistance breeding efforts.

Citrus yellow vein clearing virus is likely spread in California via plant propagation. Several important citrus pathogens are transmitted through grafting and plant propagation. The best control method for these pathogens is to use pathogen-free plant materials acquired from certified citrus nurseries, but citrus propagation in backyards and urban settings is less strictly controlled. ARS researchers in Parlier, California, and California Department of Food and Agriculture collaborators determined that approximately 25 percent of dooryard citrus in the area sampled was infected by citrus viroids (a tiny virus-like pathogen) that are transmitted solely by plant material grafting and propagation. Thirteen percent of samples collected were infected with the emerging pathogen citrus yellow vein clearing virus (CYVCV), and approximately one-third of the CYVCV-infected trees were also infected with graft-transmitted citrus viroids. The presence of CYVCV in the same trees as graft-transmitted pathogens suggests that plant propagation, along with insect transmission, is likely causing a significant amount of CYVCV spread in California. These results could be used by regulatory agencies for strengthening quarantine efforts around CYVCV-infected sites.

AGRAMP: mining antimicrobial peptides in crop genomes using artificial intelligence. Identifying potent new antimicrobial peptides (AMPs) for U.S. agriculture is critical to protecting the bioeconomy against production shocks due to rapidly evolving pathogens. However, using laboratory analysis to identify new AMPs from crop germplasm is resource-intensive and time-consuming. ARS scientists used machine learning models to develop a bioinformatics approach for predicting and selecting AMPs to use against plant pathogenic bacteria. In the citrus genome, approximately 7 percent of a 10,000-peptide dataset from the intergenic region and 7 percent of a 685,924-peptide dataset from the whole genome were predicted as probable AMPs. A subset of the predicted AMPs was successfully tested against *Spiroplasma citri*, the causative agent of citrus stubborn disease, demonstrating the predictive power of the machine learning models.

Almond is a transmission-competent host of plum pox virus. Plum pox virus (PPV) causes devastating disease in stone fruit and is widely present in most commercial stone fruit production areas except in the United States. There are conflicting reports in the scientific literature about whether almond is susceptible or may have some natural immunity to PPV. ARS researchers in Frederick, Maryland, and Kearneysville, West Virginia, demonstrated that almond can be infected by a specific isolate of PPV. Even though detected virus concentrations were approximately 100-fold lower in almond than in the more susceptible peach, they showed that aphids feeding on infected almond can then transmit PPV to peach. These results have profound implications for efforts to keep PPV out of the United States and specifically for stone fruit production in California, where almonds are often grown near peaches, plums, and apricots.

Identification of a new virus infecting melons in the Western Hemisphere. Plant viruses can cause significant economic losses to growers. In 2023, unusual symptoms were observed in melon and

watermelon plants from Yuma County, Arizona, and Imperial County, California. ARS scientists in Salinas, California, identified the causal agent as watermelon chlorotic stunt virus (WmCSV) from received samples and sequenced the genome of this virus for comparison to other global isolates. This is the first identification of WmCSV infecting cucurbit plants in the Western Hemisphere. This virus can cause significant yield loss in watermelon and is contributing to an existing virus complex on melon in the southwestern United States. As a result, the cucurbit industry has been notified of the threat posed by this virus in efforts to keep it from being transported to other regions of the United States.

Demonstration of an enzyme produced in modified yeast is active in killing a pathogen of poultry.

Necrotic enteritis (NE) is a prevalent and often fatal gastrointestinal disease in poultry caused by the bacterium *Clostridium perfringens*. NE not only impacts the health and welfare of chickens, but global losses have been estimated to cost the poultry industry \$6 billion annually. Antibiotics were commonly used for the treatment and prevention of NE. However, alternative antimicrobial solutions are necessary to address rising concerns about antibiotic resistance. In collaboration with USDA-ARS researchers in Beltsville, Maryland; Peoria, Illinois; and the University of Maryland Eastern Shore, Princess Anne, Maryland, a novel approach was developed to control *Clostridium perfringens* using an enzyme, called phage endolysin, that has the unique ability to selectively target and destroy the harmful bacterium. The enzyme, when produced in yeast, was able to eliminate up to 99.99 percent of the harmful bacteria in laboratory testing and experiments using the contents from chicken intestines. These promising results suggest that incorporating this modified yeast and, in the future, modified plant material into chicken feed could serve as an effective strategy to control this devastating disease, reducing dependency on antibiotics.

Metal containing enzymes control bacterial social interactions. Bacteria are single-celled organism that often form colonies of millions or even billions of identical copies of themselves. Bacterial colonies will sometimes coordinate their behavior and organize themselves as a group, such as when they try to protect themselves from physical or chemical stresses or acquire resources from unrelated bacteria. The mechanisms involved in formation of multicellular bacterial communities and coordination of bacterial behavior are complex, and information is lacking. ARS scientists in Ithaca, New York, discovered that some plant pathogenic bacteria can detect unrelated bacteria when they are nearby. The pathogenic bacteria respond by swimming towards the unrelated bacteria, presumably to prevent potential competitors from using up the resources. Using molecular approaches and genomic analysis, they found that the bacteria use genes involved in making a metal binding molecule for coordinating the swimming behavior. This research helps to understand why plant pathogenic bacteria can invade plant tissues and displace other bacteria. Furthermore, these results can be used to identify and assemble bacterial communities that cannot be displaced by pathogens, thereby preventing plants from being infected by aggressive pathogens.

Two fungi identified as major cacao pathogens in Puerto Rico. Cacao production is a rapidly expanding industry in Puerto Rico, with new farmers having planted approximately 20,000 trees in the past few years. To determine the etiology and extent of diseases affecting cacao in Puerto Rico, scientists from ARS in Frederick, Maryland, surveyed eight sites around the island. Although *Phytophthora palmivora* had been thought to be the primary pathogen affecting cacao in Puerto Rico, two other fungi (*Lasidiopodia theobromae* and *Diplodia tulliensis*) were found to be the most common pathogens on the island. Infection studies found that all three pathogens produced similar levels of disease on stems. *D. tulliensis* produced slightly smaller lesions on pods, which was offset by the increased prevalence of *D. tulliensis* found in over 50 percent of diseased pods in this study. This improved understanding of which pathogens are present on the island will help scientists and farmers control disease by selecting fungicides effective against both oomycetes and fungi.

First report of a virus affecting lettuce in Mexico. Annually, Mexico produces over 500,000 tons of lettuce. In recent years, high levels of disease incidence have occurred, with plants showing symptoms of virus infection. ARS scientists in Salinas, California, have worked collaboratively with the Autonomous University of Chapingo, Mexico, to determine Impatiens necrotic spot virus (INSV) as the major pathogen that has resulted in economic losses in lettuce. INSV is transmitted by a small insect, which is common in the region and also present in California. This finding has provided new knowledge on the causative pathogen and provides a baseline for developing management strategies for INSV and its insect vector.

Nematode communities can be described by DNA techniques. Nematodes are microscopic worms and are the most numerous soil invertebrate, occupying all levels in the food web, from fungal and bacterial feeders to herbivores to predators. At present, they can only be described by extracting live nematodes from the soil, and identified by morphological characters under the microscope, which takes specialized training. ARS scientists in Pullman, Washington, and in Corvallis, Oregon, in collaboration with Washington State University scientists developed a database of sequences that is specific to nematodes. This was validated with potato and wheat soils across eastern Washington and Oregon, including soils that have never been cropped. Nematodes were morphologically identified and sequenced, with a high degree of correlation between the two methods. This research opens up the possibility of more extensive use of nematode communities as indicators for soil health, especially by those not skilled in nematology taxonomy.

Compost increases the plant pathogen *Verticillium dahliae* in soil. Compost is commonly used to improve chemical and biological characteristics of soil and can suppress certain plant diseases. ARS researchers in Salinas, California, demonstrated that application of plant-based compost prior to planting lettuce increased the amount of the fungal pathogen *Verticillium dahliae*. This information demonstrates that compost can have inadvertent negative effects by increasing pathogens in soil and will be useful for growers who regularly use compost as a soil amendment.

Cellular mechanism underlying beech leaf disease elucidated. The beech leaf disease is caused by a nematode, which is rapidly spreading and poses a severe threat to American beech, a tree species that serves as an important host and food source for a wide range of birds, bears, and other wildlife. The nematode severely deforms leaves and buds, resulting in high mortality in seedlings and mature beech trees alike. The mechanism by which the nematode causes the diseases is not well understood. ARS scientists from Beltsville, Maryland, used light and scanning electron microscopy to elucidate the beech leaf disease. They revealed extensive cellular changes in nematode-infected buds and leaf tissues, which effectively captured the sequential and temporal cellular events associated with the development of beech leaf disease, and simultaneously provided an in-depth, mechanistic overview of this disease. This approach not only provided insight into the development of the nematode feeding site but also revealed several critical time periods for future molecular and physiological studies of beech leaf disease. This research is being utilized by scientists, extension agents, forest managers, and quarantine officials to establish effective measures to mitigate the spread of this nematode and control the disease by targeting vulnerable points in its life cycle.

New virus discovered in *Miscanthus* species. *Miscanthus* is a genus of perennial grasses with diverse uses including as an ornamental, for biomass, and as a breeding relative of sugarcane. ARS researchers in Beltsville, Maryland, discovered a new virus, *Miscanthus virus M* (MiVM), in a *Miscanthus* accession curated at the ARS gene bank in Miami, Florida, using genetic sequencing. The genomic organization and full-length RNA sequence allowed its relationship to other viruses within its family to be determined. Further analysis indicates MiVM represents a new genus of plant viruses. This discovery, published as a

scientific article in the journal *Archives of Virology*, adds a new virus to the list of those that merit testing in germplasm curation, exchange, and breeding programs.

Surveillance model for citrus greening disease on citrus in California. Citrus greening or Huanglongbing (HLB) is the most devastating disease of citrus worldwide. To date, HLB in California has been reported only on urban backyard citrus trees; it has not been reported in any commercial citrus groves. ARS researchers in Fort Pierce, Florida, in collaboration with scientists at the University of California, Davis and California stakeholders, developed a refined risk-based survey (RBS) to help survey for HLB in complex southern urban and suburban landscapes in California. Using the model can optimize survey efforts and allocations, depending on climate, incidence reports about the insect vector and HLB, and other factors affecting spread. The RBS is updated with new detection data and refined continuously to guide surveillance. An online interface allows the most recent RBS to be available to stakeholders.

The bacterium *Ralstonia solanacearum* is threatening Florida blueberry production. *Ralstonia solanacearum* is a quarantine bacterium that threatens the blueberry production in the United States. ARS scientists from Fort Pierce, Florida, in collaboration with blueberry growers throughout Florida, isolated this damaging bacterium from field plants, soil, and irrigation water. Genetic characterization established that at least two groups of these isolates, with different genetic lineages and host ranges, were infecting blueberry. “Arcadia,” the most preferred blueberry cultivar in the state because of its typical high-market fruit quality, was the most susceptible to this bacterium, with some growers reporting up to 90 percent loss of their blueberry plants. Discovery of infective strains of the bacterium will allow more precise screening of plant material and soil treatments to identify blueberry varieties that are resistant to or tolerant of this important plant pathogen, which will greatly benefit the blueberry industry in Florida and elsewhere.

The distribution of a soilborne pathogen in California. Verticillium wilt, caused by the fungus *Verticillium dahliae*, is one of the most devastating soilborne diseases of lettuce. Within the global population of *V. dahliae*, three different races and one unknown race have been identified based upon differential interactions on tomato or whether or not isolates carry marker genes for specific races. ARS researchers in Salinas, California, in collaboration with researchers at the University of California-Davis and the University of Florida, characterized the distribution of the different races of the pathogen in Coastal California and evaluated Verticillium wilt resistance to the identified races. Knowledge of both disease resistant cultivars and the distribution of the races of *V. dahliae* is being used by growers to combat Verticillium wilt of lettuce.

Component 3. Plant Health Management

Cotton germplasm lines resistant to root-knot and reniform nematodes. Plant-parasitic nematodes (e.g., reniform and root-knot nematodes) are responsible for high yield losses in cotton. During the past 20 years, U.S. cotton farmers have lost around \$250 million annually (more than all other cotton diseases combined) because of these pests. They can also interact synergistically with root rot pathogens, including those that cause Fusarium wilt, to exacerbate disease severity. Repeated applications of pesticides and crop rotations can reduce nematode damage, but the development and use of nematode-resistant cultivars is widely considered to be the most effective, economical, and sustainable approach for managing nematodes in cotton. ARS researchers in College Station, Texas, in collaboration with Cotton Incorporated and academic colleagues, developed and registered three nematode-resistant germplasm lines with greater yields and increased fiber length and strength, compared to several elite commercial cultivars. These lines are stacked with two root-knot resistance genes and two reniform resistance genes. The lines also exhibited significant reduction in Fusarium wilt

severity. The registration and availability of these lines is already facilitating work by breeders to develop new commercial varieties that are resistant to nematodes.

Three resistance genes protect commercial corn varieties from maize dwarf mosaic disease. Maize dwarf mosaic (MDM) disease is one of the most important virus diseases of maize worldwide. Several potyviruses cause MDM but the most common ones are maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV). Despite being widespread, little is known about MDMV susceptibility in commercial field corn varieties or how MSM affects U.S. corn yields. ARS scientists in Wooster, Ohio, tested 78 high-yielding commercial field corn varieties and determined that all were susceptible to SCMV, and just one was susceptible to MDMV. Furthermore, among the susceptible varieties, yields were reduced an average of 10 percent and by nearly 30 percent in some varieties when inoculated with SCMV. Growing corn with all three major potyvirus resistance genes was found to eliminate disease symptoms, suggesting that incorporating these three potyvirus resistance genes into commercial varieties would be a viable management strategy for reducing MDM impacts on U.S. corn yields.

Development, transfer, and release of two improved soybean germplasm lines with tolerance to mature seed damage. Soybean is a global crop and a major source of plant protein for both human and animal nutrition. Mature seed damage (MSD) caused by mold, insects, heat, sprouting seed, and mycotoxins can reduce grain quality and reduce domestic and international sales and grower profits. ARS researchers in Stoneville, Mississippi, developed and publicly released two soybean germplasms that exhibit less MSD. Using these lines, university partners developed multiple new breeding lines and plan to release high-yielding cultivars with improved tolerance to MSD. The improved cultivars will be valuable to soybean producers, increase profits, and reduce the amount of damaged grain produced and sold.

Crickets can convert mycotoxin-contaminated grain into a safer product. Livestock that consume mycotoxin-contaminated grain can become seriously ill and even die. A team of ARS researchers in Stoneville, Mississippi, and academia evaluated whether crickets could safely consume mycotoxin-contaminated corn grain and if the crickets that consumed the corn could later be used as a protein supplement in livestock feed. They found crickets that consumed mycotoxin-contaminated corn and subsequently were processed as insect meal provided nearly three times the protein content of high-protein corn and reduced the mycotoxin content in insect meal by 96 percent. Livestock stakeholders are interested in using crickets to improve feed quality and reduce the waste of contaminated corn, and the cricket farming industry has requested information on improving cricket safety and improving their market value. These findings could give producers an option for preventing economic losses by using contaminated corn, let insect-livestock stakeholders safely access cheaper and previously undesirable agricultural waste streams for cricket rearing substrates, and provide livestock users with a new and safe protein feed supplement.

Seed fumigation prevents disease transmission. Seedborne plant pathogens cause billions of dollars in annual losses in the United States. The pathogen that causes spinach downy mildew, *Peronospora effusa*, is seed transmitted. ARS researchers in Salinas, California, determined that nitrogen dioxide fumigation eliminated seed transmission of spinach downy mildew from infested seed. Additional evidence indicated that the seed treatment reduced the viability of the *Verticillium* wilt pathogen, *Verticillium dahliae*, on spinach seeds. The fumigation treatment is being evaluated by the seed industry to eliminate or reduce seedborne pathogens of spinach.

Discovery of *Trichoderma* strains for effective biological control of grapevine trunk diseases. Grapevine trunk diseases caused by fungal pathogens are a significant problem for grape. Chemical control options for fungal pathogens are limited and unsustainable in many regions due to human health and environmental concerns. Biological control agents provide an important alternative to

chemical pesticides due to their ability to provide long-term, sustainable disease control. ARS researchers in Parlier, California, discovered novel *Trichoderma* species of fungi from California vineyards and tested their biological control activity to manage grapevine trunk diseases. Six of these new biological control strains provided disease control equivalent to a commercial fungicide and were able to survive in grapevines throughout the entire growing season. These new *Trichoderma* strains provide an effective biopesticide option for grape growers in California to control grapevine trunk diseases.

Genetic mapping of sunflower basal stalk rot resistance derived from the wild sunflower relative *Helianthus petiolaris*. The destructive fungal pathogen *Sclerotinia sclerotiorum* can cause three distinct diseases on cultivated sunflower: head rot, basal stalk rot, and mid-stalk rot. Basal stalk rot begins as a root infection and is unique to sunflower. ARS scientists in Fargo, North Dakota, and colleagues at North Dakota State University and University of Nebraska introduced improved basal stalk rot resistance into cultivated sunflower through a cross with the wild sunflower species *Helianthus petiolaris*. Genetic analyses identified 14 chromosomal regions associated with basal stalk rot resistance, of which 11 regions were derived from *H. petiolaris*. Genetic markers for resistance derived from *H. petiolaris* will facilitate breeding for improved basal stalk rot resistance in cultivated sunflower.

Gene for resistance to soybean rust disease identified. The United States is the second largest producer and exporter of soybeans, valued at over \$30 billion annually. Soybean rust is a devastating disease that negatively impacts soybean production throughout the world. While most soybean germplasm is susceptible to the disease, seven genetic regions have been identified that provide resistance. To identify a resistance gene called Rpp3, scientists from ARS in Ames, Iowa, and Frederick, Maryland, in collaboration with Iowa State University sequenced candidate genes from two resistant soybean lines. Five Rpp3 candidate genes were identified in each resistant line, but only one was identical in both lines. Further analysis suggests that a single Rpp3 candidate gene, Rpp3C3, is responsible for Rpp3-mediated resistance. This research will allow soybean breeders to rapidly differentiate between known and potentially novel variants of Rpp3, facilitating the breeding of resistance.

***Xylella fastidiosa* strains from different growing regions cause similar Pierce's disease severity.** *Xylella fastidiosa* (Xf) is a bacterial pathogen that causes deadly Pierce's disease of grapevines in California. Hundreds of Xf strains were collected from vineyards in California in the last few years, but there was no information about disease severity caused by most of these strains. ARS researchers in Parlier, California, compared disease severity in plants infected with 71 different Xf strains from all California grape-growing regions and found no significant difference in disease severity between different strains. This research provides important information for managing Pierce's disease in vineyards because it shows that all pathogen strains cause the same amount of disease and can be managed in a similar way.

Vine mealybug life stage determines survival following insecticide treatment. Vine mealybug is the number one pest of grapevine in California. Control of this pest relies on applications of systemic insecticides, and overreliance on a few active ingredients resulted in reduced efficacy of these products. Insects can reduce chemical exposure by not feeding on treated plants, and it is unclear whether observed insecticide failure is due to higher tolerance to the chemical, or reduced feeding by the insects under certain conditions. To better understand failures of chemical control products, ARS researchers in Parlier, California, tested vine mealybugs in the laboratory for susceptibility to the systemic neonicotinoid insecticide imidacloprid. High concentrations of imidacloprid applied to grapevines did not kill mature female vine mealybugs or prevent them from producing viable eggs, whereas low concentrations of imidacloprid did kill newly hatched insects. This information is important for understanding insecticide efficacy for controlling vine mealybugs and is expected to improve insecticide application strategies in vineyard management.

Development of a new method to measure plant defense compounds in cotton. Jasmonic acid (JA) and salicylic acid (SA) are primary compounds involved in the natural plant defense systems against pathogens, including nematodes. However, published methods for measuring JA levels in plants were found to be unsuccessful for measuring the levels in cotton. ARS researchers in Tifton, Georgia, in collaboration with the University of Georgia developed new methods for measuring JA levels in cotton. This new method will allow research into the role of the SA and JA plant defense systems in host-plant resistance and pathogen interactions in cotton, including the interaction of nematodes and a fungal pathogen in Fusarium Wilt disease of cotton.

Fighting fungus with fungus to control blue mold decay. Blue mold causes economic losses, increases food waste, and results in patulin contamination for processed fruit products. The disease is caused by a fungus, affects apple and pear fruit during storage, and new methods of control are needed. Researchers in Beltsville, Maryland, have demonstrated that the fungus *Penicillium chrysogenum*, can inhibit and reduce blue mold decay severity and incidence in apple fruit. Before this work was conducted, scientists did not know the exclusionary impact between these two different species and that *P. chrysogenum* could block decay development in different apple fruit cultivars. This breakthrough represents a new paradigm for biological control and provides a platform to release these strains for chemical-free, environmentally friendly postharvest blue mold control.

New weapons to fight tar spot disease in corn. Tar spot disease of corn can cause significant yield losses when environmental conditions are conducive to spread of this fungus in the midwestern United States. Biological control is an approach that uses natural microbes to inhibit or kill plant pathogens, which provides a more sustainable method for farmers to protect their crops. ARS researchers in Peoria, Illinois, isolated two strains of the bacterium, *Priestia megaterium*, from corn that has potential biological control properties. These bacterial strains were grown in large quantities in the laboratory, and a portion of each strain was applied to corn seeds that were then sown in field plots. Two years of repeated field trials resulted in successfully reducing disease incidence when the two bacterial strains are applied as a seed coating. These bacteria could be utilized in pest management strategies to reduce the impact of tar spot disease in corn.

Improving fungicide performance against Phytophthora root rot of red raspberry. Washington state is the largest producer of frozen red raspberries in the United States, generating \$112 million annually. However, Phytophthora root rot disease threatens production of this valuable crop. Growers typically apply fungicides during cool fall weather to manage this disease. Despite this, the treatments often do not provide adequate control, leading growers to suspect fungicide resistance had developed. However, ARS researchers in Corvallis, Oregon, in collaboration with Washington State University researchers found no evidence for fungicide resistance. Instead, they discovered that the timing of the fungicide applications may be the problem and suggested that applying the fungicides during the summer could improve disease control significantly.

Discovery of new durable resistance to stripe rust in wheat. Stripe rust disease causes yield losses up to 40 percent. Stripe rust is best controlled through growing resistant varieties. ARS scientists in Pullman, Washington, identified a new gene conferring high-temperature adult-plant (HTAP) resistance to stripe rust in wheat. HTAP resistance, derived from wheat relative, *Aegilops comosa*, was effective against the current U.S. wheat stripe rust population while the wheat line carrying the Yr8 gene was ineffective. The lines with HTAP resistance and the identified molecular markers associated the resistance are useful for breeding programs to develop new elite wheat varieties with durable stripe rust resistance in the United States.

Identifying “broken genes” in corn to improve pest resistance. Corn is one of the most important grain crops in the world and its production can be severely limited by fungal pathogens and insect pests.

Breeding corn plants with improved resistance to pests and pathogens has been helpful, and multiple genes appear to be involved in both insect and pathogen resistance. During the breeding process, mutations can unintentionally be introduced into these resistance genes and reduce their effectiveness. ARS researchers in Peoria, Illinois, identified mutations in a group of pest resistance genes that make them less effective. Identifying these problematic genes allows them to be removed from breeding stock or have them repaired. This discovery will allow corn breeders to produce plants with better pest resistance and on farm performance.

An identified chromosome in allohexaploid wheat contains genes encoding herbicide-detoxifying enzymes. Allohexaploid wheat (*Triticum aestivum* L.) achieves tolerance to many synthetic plant hormone herbicides, including halauxifen-acid (HA), through enhanced detoxification. While the detoxification pathway of HA in wheat has been characterized, the genes encoding the detoxification enzymes have yet to be identified, which would be useful for breeding for herbicide tolerance or developing *in vitro* assays for herbicides. ARS researchers in Pullman, Washington, in collaboration with scientists at the University of Illinois, measured the abundance of HA, over time in wheat lines with an abnormal number of chromosomes via liquid chromatography-mass spectrometry, and found that lines lacking chromosome 5A consistently accumulated significantly more HA. Chromosome 5A was found to contain genes encoding HA-detoxifying enzymes, and the lack of these genes results in higher accumulation of HA relative to lines that maintain 5A. This finding could facilitate future development of wheat resistant to plant-hormone based herbicides, which would provide growers another tool in managing weeds that have become resistant to other herbicide classes.

Searching for resistance to Red Leaf Blotch of soybean. Red Leaf Blotch (RLB) is devastating to soybeans in certain areas of Africa, and resistance of locally grown soybean varieties is almost non-existent. To identify candidate genetic regions associated with resistance to the fungal pathogen that causes RLB, ARS researchers in Urbana, Illinois, are using phenotypic data from field, greenhouse, and laboratory assays from Ethiopia and Nairobi and performing genome wide association analysis among the screened germplasm from the USDA Soybean Collection. These data are being used to identify germplasm with these genetic regions which will then be incorporated into suitable soybean lines for the African growing regions. Characterizing the pathogen and identifying genetic resistance before the pathogen appears in the Western hemisphere will ensure that soybean producers in the United States can minimize the economic impact of this serious disease.

Discovery of bacteriophages for biocontrol of potato bacterial soft rot disease. *Pectobacterium carotovorum* is an economically important bacterium causing the destructive bacterial soft rot disease in many ornamental, fruit, and vegetable crops worldwide, particularly potatoes. This plant pathogen is very difficult to control. Bacteriophage therapy is a promising and environmentally safe alternative to pesticide use to combat bacterial diseases either in the field or during storage. ARS scientists in Beltsville, Maryland, in collaboration with scientists in Egypt, discovered and determined the genome sequences of two novel bacteriophages (designated PcaP1EGY and PcaP2EGY) isolated from a potato field in Egypt. These phages specifically target *Pectobacterium carotovorum* strains, displaying visible destructive activity of the bacterium and significantly protecting both potato tubers and plants from bacterial soft rot disease. Their potential as biocontrol agents against this damaging disease has been shown promising under field conditions or during post-harvest storage and will greatly benefit the potato industry worldwide.

Differentially expressed genes identified in resistant cotton accessions challenged by reniform nematode. Reniform nematode is a threat to United States cotton production and causes hundreds of millions of dollars in loss each year. Current control practices using pesticides and crop rotation do not provide season-long suppression of the reniform nematode population; growing resistant cultivars is a

more economical and effective way to control this important parasite. However, resistance to reniform nematode has not been found in the widely grown upland cotton (*Gossypium hirsutum*) but is found in its relatives, Sea Island cotton (*G. barbadense*) and Asiatic cotton (*G. arboreum*). Two resistant lines each of Sea Island cotton and Asiatic cotton were used by ARS researchers in Stoneville, Mississippi, to study what genes were activated in these lines in response to infection by reniform nematodes. Two genes previously reported to provide resistance to a different nematode species, along with other genes that have potential roles in plant defense, were identified. This work adds to our basic knowledge about how the resistant plants react to the nematode. The findings will help scientists develop genetic markers for the resistance genes that cotton breeders can use to develop resistant cultivars for cotton growers.

A new source of genetic resistance to tomato brown rugose fruit virus. Tomato brown rugose fruit virus (ToBRFV) is an emerging and devastating virus that poses a serious threat to the U.S. \$2.5 billion tomato industry and is also very damaging to pepper crops. Because of ToBRFV quarantine status, USDA issued a federal order in 2019 and is still regulating the movement of tomato seeds and propagation plant materials because of the virus, which has so far been documented in several countries. All available commercial tomato cultivars are susceptible to the ToBRFV, so it is important to identify sources of resistance to the virus. ARS scientists in Charleston, South Carolina, screened the USDA tomato germplasm collections and identified several accessions of *Solanum pimpinellifolium*, a tomato relative, that show resistance to ToBRFV. *Solanum pimpinellifolium* could serve as a new source of resistance to ToBRFV and can be easily crossed with tomato to develop new cultivars with resistance to the virus. This discovery will greatly benefit the tomato industry in the United States and elsewhere.