

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

National Program 303



PLANT DISEASES

FY 2023 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'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.

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 45 research projects located in 23 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 2023 these funds were distributed across 35 competitive projects to ARS scientists and their collaborators. These projects included diseases that affect alfalfa, almond, avocado, beech, blueberry, cacao, citrus, coffee, cotton, grapevine, maize, oak, olive, palm, peach, peanut, plum, potato, rice, sorghum, sugarcane, tomato, watermelon, and wheat.

During FY 2023, NP 303 scientists participated in research collaborations with scientists from Argentina, Australia, Austria, Azerbaijan, Belgium, Bolivia, Brazil, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Egypt, Ethiopia, France, Germany, Ghana, Greece, Guatemala, India, Israel, Italy, Japan, Jordan, Kenya, Lithuania, Mexico, Morocco, Netherlands, New Zealand, Nigeria, Philippines, Poland, Portugal,

Saudi Arabia, South Africa, South Korea, Spain, Sri Lanka, Switzerland, Taiwan, Tanzania, Tunisia, Turkey, and the United Kingdom.

The quality and impact of NP 303 research was evidenced during FY 2023 by the following:

- 217 refereed journal articles published,
- 3 new invention disclosures (patent only) submitted,
- 12 variety and/or breeding lines released which were evaluated and/or bred for disease/pest resistance, and
- 3 new patent applications filed.

NP 303 encompasses the following three research components in fiscal year 2023:

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 2023, 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

Diagnostic tools for a corn virus affecting trade issues. High Plains wheat mosaic virus infects wheat, maize, and other cereals and is widespread in the United States, especially in the Midwest and Pacific Northwest where corn seed is produced. Recently imposed trade restrictions prevented exporting corn seed infested with the virus, impacting the ability of U.S.-based seed companies to export seed to key trading partners. Reliable diagnostic tests for the virus are urgently needed to test and certify corn seed that is clean of the virus and safe to export; such a tool that could potentially affect billions of dollars in trade. ARS researchers in Wooster, Ohio, developed five new diagnostic assays for High Plains wheat mosaic virus that can detect wide variants of the virus. These assays are being used by university and industry researchers and will benefit diagnostic labs that test for the virus, corn seed industry stakeholders that depend on these test results, and regulatory authorities that certify seed as safe for export, facilitating safe seed trade with other countries.

Using machine learning to assess the genetic diversity of the North American citrus greening pathogen. Huanglongbing (HLB), or citrus greening, is a highly destructive citrus disease associated with the nonculturable bacterium *Candidatus Liberibacter asiaticus* (CLas). Understanding the population

diversity of CLas is critical for HLB management. ARS scientists in Parlier, California, and collaborators from Mexico collected 40 CLas samples from 20 states in Mexico and analyzed them using a machine learning approach. Results showed there were at least two different introductions of CLas into Mexico, and that CLas strains from Mexico and the United States are closely related. Results can inform efforts to manage HLB in North America by improving understanding of how HLB has historically moved around the continent, which will be useful information for USDA's Animal and Plant Health Inspection Service and other regulatory agencies.

Screening for sugar beet resistance to the crown and root rot pathogen. The soilborne pathogen *Rhizoctonia solani* causes sugar beet crown rot and root rot diseases, which are both serious threats to sugar beet production and processing. Before the adoption of Roundup Ready® sugar beet (RRSB) cultivars, crown rot was a serious problem resulting from mechanical tillage operations required for weed control. Following the introduction and large-scale cultivation of RRSB, crown rot was reduced but root rot became severe, so screening methods for developing *Rhizoctonia*-resistant cultivars needed reassessment. ARS scientists in Beltsville, Maryland, and North Dakota State University scientists evaluated crown inoculation and root inoculation methods for developing *Rhizoctonia* root rot and assessed the efficacy of distinguishing how the pathogens affected sugar beet cultivars. Results, which were published in the journal *Crop Protection*, demonstrated that the root inoculation method is optimal for greenhouse assessments of disease in sugar beet germplasm, and that using this method is convenient and accurate for screening RRSB cultivars in a resistance breeding program.

Foreign variants of corn pathogen may threaten U.S. crops. The United States is the largest producer, consumer, and exporter of corn in the world, with annual production valued at more than \$82 billion. U.S. growers minimize damage to corn caused by anthracnose disease by planting corn varieties that are resistant to the pathogen populations found in North America. However, outside of North America, corn anthracnose is very difficult to control, and the disease is becoming increasingly widespread. An international team of scientists, including ARS researchers in Frederick, Maryland, compared the DNA profiles of anthracnose pathogens collected worldwide and published their results in *mBio*. The research showed that DNA profiles varied depending on the continent where the pathogen was collected, and that U.S. isolates have different DNA profiles from foreign isolates. Developing effective crop resistance to disease requires knowing a pathogen's genetic structure, so findings from this research guide efforts to breed corn with resistance to variants of the anthracnose pathogen residing in the United States and globally.

A new virus infecting pear germplasm. Several viruses infect pear and can cause virus diseases that are difficult to control. Virus infections in curated germplasm accessions, especially those imported before quarantine regulations were applicable, are not well studied. ARS scientists in Beltsville, Maryland, tested eight pear accessions in the National Plant Germplasm System genebank in Corvallis, Oregon, that had symptoms of viral infection. Bioinformatic analyses from high-throughput sequencing revealed the presence of a new virus belonging in the genus *Partitivirus*, which is known to be associated with symptomless infections. The nearly full-length sequence of the virus genome was determined and published in *Frontiers in Microbiology*, and a PCR-based detection protocol was developed. Determining the infection status of clonal germplasm accessions will support better curation practices, including the possible application of treatments to minimize risks associated with distributing infected material.

Biological features that contribute to the spread of endemic viroid diseases in crops. Worldwide yield losses caused by viroids (small noncoding infectious RNAs) can reach 17-64 percent, depending on the viroid strain and plant crop species. Viroids found in Mexico exhibit considerable biodiversity. An ARS

scientist in Beltsville, Maryland, and colleagues in Mexico highlight biological features of viroid species endemic to Mexico, including in tomato, citrus, and avocado. Their published report in the scientific journal *Cells* includes studies on the genetic relationships among strains, their economic impact, and geographical distribution; it also discusses epidemiological features, including a broad host range and possible long-distance, seed, or insect-mediated transmission. These findings provide valuable insights for research scientists, growers, and regulatory agencies about the biology of viroid diseases and can help in the development of programs to control the introduction, movement and spread of viroids to avoid economic losses in agricultural industries.

One step closer to soft rot resistant potato. Soft rot diseases cause approximately one billion dollars of damage annually to potato worldwide. Beyond cultural practices, like using certified seed and sanitation, there are few management options since all commercial varieties of potato are susceptible to soft rot. ARS scientists in Ithaca, New York, and Sturgeon Bay, Wisconsin, identified the parts of three potato chromosomes that help protect potato tubers from soft rot disease caused by bacteria. These results, published in *Frontiers in Microbiology*, will enable researchers to find and carry the resistance trait. Knowing the parts of the chromosome that carry soft rot disease resistance is a needed step to transferring resistance to protect cultivated potato.

Changes in *Xylella fastidiosa* DNA provides new insight into DNA rearrangement. *Xylella fastidiosa* (Xf) infects hundreds of plant species and causes severe disease in several important crops including grape, almond, citrus, and olive. Specific strains of Xf will cause disease in only some host plant species, but the mechanism for the host specificity cannot always be determined based on DNA sequence alone. ARS researchers in Parlier, California, identified DNA methylation patterns present in a wide range of Xf strains and found that closely related strains maintained similar patterns. This suggests that DNA modification patterns could prevent recombination between less closely related strains, which is important for understanding the emergence of new strains and Xf adaption to new hosts. This will benefit regulators such as the USDA Animal and Plant Health Inspection Service by allowing more informed decisions regarding which Xf strains require increased scrutiny and the likelihood of new strains emerging.

Using genetic information of citrus yellow vein clearing virus to study its origin. Citrus yellow vein clearing virus (CYVCV) is an emerging exotic disease in California, that poses a threat to the citrus industry in the United States. In other parts of world, CYVCV infects trees through wounding, grafting, and by aphids and whiteflies. Most citrus cultivars are susceptible but do not show symptoms. However, symptoms are very distinct in lemons and infections cause significant loss of production. CYVCV was detected in 2022 in urban citrus trees in Tulare, California. ARS researchers in Parlier, California, sequenced the entire genome of the California strain of CYVCV. Comparing genetic information from other strains collected around the world revealed that the closest strain is from India. This suggests that the CYVCV-CA1 strain was introduced to California by infected plants or budwood. Results will be useful for USDA Animal and Plant Health Inspection Service, Plant Protection & Quarantine and California Department of Food and Agriculture to target entrance pathways of these exotic pathogens and raise awareness about potential high-risk populations.

A new molecular tool protocol optimized for discovering new viruses infecting multiple hosts. Citrus leprosis virus (CiLV) causes a destructive emerging viral disease of citrus in the Americas. Genetic diversity of the virus genome associated with the citrus leprosis disease complex has complicated current detection and diagnostic measures. An important tool for the detection and discovery of plant viruses is high-throughput sequencing (HTS) that enables scientists to read millions of DNA fragments

more quickly and economically. ARS scientist in Beltsville, Maryland, adopted an HTS protocol for virus detection that was optimized utilizing three plant species that are known hosts of CiLV. Although the HTS protocol was evaluated to detect several known plant viruses in the three studied hosts, multiple new hosts in nature were identified that were infected with CiLV. Furthermore, the current protocol, which was described in *Frontiers in Plant Science*, was also successfully used to identify four new virus species of carlavirus, one potexvirus, and one potyvirus in hibiscus. Overall, using this new HTS method will enhance the surveillance of regulated viruses by accurately detecting known viruses and identifying novel viruses infecting known or new host species.

Demonstrating that dogs can detect viruses. Early detection and rapid response is the key to mitigating all plant diseases. Detector dog development and deployment provides early pathogen detection and rapid response and offers the possibility to mitigate plant diseases before they become epidemic. Rather than relying on traditional field scouting, ARS researchers in Fort Pierce, Florida, and industry cooperators initiated a program to cross-train detector dogs for squash vein yellowing virus (which causes watermelon vine decline) and tomato chlorotic spot virus in tomato and pepper. After assessing results for accuracy, sensitivity, and specificity, the researchers found the dogs detected both viruses as effectively as field scouting. Public demonstration of canine detection at a well-attended public stakeholder event provided an ideal demonstration of this concept. A proof-of-concept has also been demonstrated for canine detection of viruses in wine grapes, and a major strawberry producer in California has initiated a project for using canines for pest detection in strawberry nursery production.

High genetic diversity of red leaf blotch pathogen of soybean in Africa. Red leaf blotch (RLB) is an important emerging soybean disease confined to Africa, but very little is known about the pathogen. At the start of this project in the summer of 2022, there were only three isolates of the RLB pathogen (*Coniothyrium glycines*) at an international research center and 12 isolates in storage in the United States that were collected decades ago. Therefore, little was known about the genetic diversity of the pathogen across Africa even while it has become increasingly prevalent and yield limiting. ARS scientists in Urbana, Illinois, now have more than 130 purified isolates spanning six countries in their collection. They were able to genetically characterize 108 of them and found a high level of diversity that neatly correlated with the different geographic regions where they were collected. These results show that each separate geographic area contains its own unique population of the pathogen, suggesting that the pathogen must not be spreading readily over large distances. These types of discoveries will help with RLB management and may help mitigate the spread of RLB.

Detection methods for identifying emerging tomato virus. Tomato torrado virus (ToTV) is a virus of concern for tomato production, causing fruit and leaf necrosis and loss of crop and seed production. The virus is not present in the United States but could be introduced via plant material, so it is critical to develop methods to detect the virus at ports of entry. ARS scientists in Salinas, California, and Corvallis, Oregon, designed methods to detect all genomic variants of ToTV currently known and focused on detecting genes encoding the virus coat protein. Methods did not cross-react with related members of the genus torradovirus based on computer alignment evaluations using the genomic sequence of 35 torradovirus species available in the public genome database. This method is being delivered to USDA Animal and Plant Health Inspection Service for use in identifying ToTV in imported plant material to protect U.S. tomato fruit and seed production.

Understanding the genetic diversity of viruses affecting vegetable and horticultural crops. Impatiens necrotic spot virus (INSV) affects vegetable and horticultural crops globally, including lettuce production in California. ARS researchers in Salinas, California, and scientists at Washington State University,

sequenced several genomes of INSV from samples collected throughout the United States. These results demonstrate that INSV isolates currently impacting lettuce production in the western United States are very similar to historical isolates, indicating that the current epidemic is not driven by a change in the virus population itself, but by other causes.

New DNA sequence resources for *Xylella fastidiosa* established. *Xylella fastidiosa* (Xf) is an economically important bacterial pathogen of many crops. Knowing the complete genetic information is a critical resource for understanding Xf biology. ARS scientists in Parlier, California, Byron, Georgia, and researchers at University of Georgia used next-generation sequencing technology to create DNA genetic resources for Xf strains causing phony peach disease and blueberry bacterial leaf scorch disease. This genomic information filled gaps in Xf genetic databases, which are critical for genetic and taxonomic studies of Xf. Researchers and regulatory agencies such as USDA Animal and Plant Health Inspection Service benefit from sequence data to improve diagnostic technologies.

Identifying genetic diversity among rose rosette virus. Roses are one of the most widely cultivated ornamental plants in the world. Rose Rosette Disease has caused significant economic losses in the United States over the last several decades, spreading from wild roses and the invasive multiflora rose into cultivated roses. Rose rosette virus (RRV) is the causal agent, and very few commercial rose varieties are resistant to RRV infection. ARS researchers in Beltsville, Maryland, and Texas A&M University collaborators conducted a genomic study to better understand RRV genetic diversity, population structures, and the nature of the genetic changes among the complete genomes of 95 sequenced RRV isolates. They found differences within each genome segment among isolates and evidence of genome segment reassortment. Several viral proteins also appeared to evolve independently, suggesting that diversity may be important for adapting to new host genotypes and plant resistance genes. These results suggest greater diversity among RRV isolates than previously recognized and suggest new avenues for disease management, including breeding for long-lasting RRV resistance in roses.

New purple coneflower pathogen. Downy mildew diseases of ornamentals have recently emerged as important and deadly pathogens. ARS scientists in Beltsville, Maryland, and university scientists in Germany recently discovered a previously unknown pathogen on coneflower, an important ornamental and medicinal plant. The identification of this downy mildew pathogen is also the first step towards developing control measures that can be specifically targeted against the pathogen and prevent millions of dollars of damage to nursery crop growers. These findings were described in the scientific journal *Fungal Systematics and Evolution*.

New and more virulent wheat-stem-rust-pathogen strains in Idaho. Protecting the U.S. wheat crop from diseases is necessary for producing high-quality wheat to meet U.S. demands and for the United States to continue as a global leader in wheat exports. Emerging strains of the wheat stem rust pathogen threaten U.S. wheat production. ARS researchers in St. Paul, Minnesota, identified six wheat stem rust pathogen strains from a single sample from Idaho Falls in mid-July 2022. The virulence of these strains was unusual and included virulence to resistance genes currently used to protect U.S. wheat from strains of the stem rust pathogen. In addition, the samples came from the alternate host of stem rust, barberry, indicating the importance of monitoring the alternate host in disease surveillance. This finding supports wheat breeders in the United States by directing efforts in selecting for stem rust resistance to additional strains of the stem rust pathogen now present in the United States.

Nematode responsible for beech leaf disease now found in Michigan. The beech leaf disease (BLD) nematode is a newly emergent nematode species that causes severely deformed leaves and buds and mortality in seedlings and mature beech trees in North America. Beech leaf disease has been spreading steadily from Ohio to 10 additional states since 2012. ARS scientists in Beltsville, Maryland, and Michigan State University collaborators used morphological features and DNA markers to identify this nematode for the first time in the state of Michigan. This discovery provides vital information about the expanding range of this pest to scientists, growers, action agencies, and extension agencies involved with controlling its spread.

DNA information recovered from old nematodes provides new insights. The USDA Nematode Collection (USDANC) is currently one of the largest nematode collections in the world. For years, it has not been possible to recover intact DNA from collection specimens that are typically preserved in a DNA-damaging fixative called formalin. ARS researchers in Beltsville, Maryland, successfully extracted DNA from formalin-fixed nematodes, allowing comparison of current isolates with decades-old collection specimens to confirm the identity of a stunt nematode called *Sauertylemchus maximus* from Arlington, Virginia. This discovery is significant because it represents the first report of this nematode from Virginia and the first report of successful DNA extraction from fixed nematode specimens deposited in the USDANC. This research opens more opportunities for ARS scientists to re-examine material from the collection to shed new light on current nematode discoveries.

Genomic characterization and distribution of a newly described virus infecting blueberries. The U.S. commercial blueberry industry value is more than \$1.1 billion. Perennial blueberry plants are grown in the field for several decades, which increases their susceptibility to viral infections that may cause significant losses. An ARS scientist in Corvallis, Oregon, collaborated with the University of Arkansas to complete the genomic sequence and characterization of a new blueberry virus, Blueberry Virus L, a luteovirus in the family Tombusviridae. Field surveys indicate this virus is widespread throughout the United States, including the Pacific Northwest, and its incidence ranges from around 5 to 80 percent, depending on plant age, variety, and location. These results were published in the journal *Virus Research*. The genetic information will be applied to develop and optimize virus detection in blueberries.

New tools to differentiate cool-virulent strains in *Ralstonia solanacearum* species complex. *Ralstonia solanacearum* race 3 biovar 2 (r3b2) strains can cause a devastating brown rot disease of potato under cool temperature conditions, a phenotype termed cool virulence. As a result, they are listed as select agent pathogens in the United States and are subject to strict government quarantine and security responses. Currently only one PCR assay, developed previously by ARS researchers in Beltsville, Maryland, is available to identify the critically important cool virulence region for specific and accurate detection of the r3b2 strains. These scientists recently found that the gene *ripS1* plays a role in the cool-virulence phenotype of r3b2 strains. They used this gene to develop an assay that can separate cool virulence strains, including r3b2, from non-cool virulence strains of *R. solanacearum*. When a sample tests positive for r3b2 by the original PCR assay, regulatory agencies can use this new test to confirm the presence of the cool-virulence-related *ripS1* region in the samples to increase the confidence of detecting the cool-virulent select agent pathogen.

A new species of virus-transmitting needle nematode in Maryland. Needle nematodes of the genus *Longidorus* are a major group of plant pathogens with a wide distribution and host range, and they sometimes are vectors of plant pathogenic viruses. A team of ARS scientists in Beltsville, Maryland, and a colleague from California described a new species of needle nematode that was discovered in two diverse environments: tall fescue grass growing along the bank of the Patuxent River in Maryland, and

near a walnut tree in California. Anatomical and DNA traits described for the new species are essential for future identifications of this needle nematode by scientists, diagnosticians, action, and extension agencies involved in nematode research and control.

New and emerging water mold causes plant diseases in subtropical areas. Safeguarding U.S. agriculture and landscapes requires rapid insight into new plant disease risks. To improve U.S. preparedness for plant disease threats, ARS researchers in Frederick, Maryland, collected, identified, and tested water molds collected from countries outside the United States, including those from citrus production areas. New variants of the 'plant killer' group *Phytophthora* were the initial target of the collection. These 'plant killers' average 6 percent losses to Florida citrus, reducing crop value by \$26 million. Surprisingly, most of the water molds common to citrus were in a new group, *Phytopythium*. To understand their impact on citrus, the scientists used greenhouse trials to compare the new foreign water molds to U.S. citrus-damaging water molds. The foreign water molds caused up to twice as much root rot as the well-known, domestic-origin water molds. While the new water molds undergo more risk tests, Florida citrus soils are being surveyed to connect U.S. water mold presence and abundance to levels of citrus root rot.

New detection methods for pear leaf scorch disease pathogen. *Xylella taiwanensis* (Xt) is a bacterial pathogen that causes pear leaf scorch disease (PLSD) in Taiwan. The pathogen is a quarantine pathogen and has not been detected in the United States. Accurate and sensitive Xt detection is critical for disease management and for international quarantine and pathogen surveillance efforts. ARS scientists in Parlier, California, and collaborators from Taiwan developed five Xt-specific molecular detection assays. These detection assays will facilitate the current disease management efforts in Taiwan and contribute to pathogen surveillance around the world where Xt introduction is a concern, including the United States.

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

AI-powered crop protection. Crop pathogens exhibit a remarkable ability to change and adapt, which enables them to overcome extant resistance genes in crop varieties and evade control strategies and chemicals that were once effective. ARS scientists in Beltsville, Maryland, and George Mason University scientists developed new machine-learning models to predict and select novel antimicrobial peptides (AMPs) against plant pathogenic bacteria. AMPs are promising alternatives to traditional antibiotics for combatting plant pathogenic bacteria in agriculture and the environment. The models were applied to predict putative AMPs encoded by DNA sequences of unknown functions in the citrus genome. Several of these predicted AMPs were tested in the laboratory and were observed to have growth-inhibitory effects on *Spiroplasma citri*, a bacterial pathogen of citrus trees. This accomplishment provides a valuable tool for identifying and selecting potential AMPs and contributes to developing effective strategies for plant disease management.

Improved weed management to combat an insect-transmitted virus affecting lettuce. Annual lettuce production in the California Salinas Valley accounts for more than 70 percent of total U.S. lettuce production. However, since 2019, impatiens necrotic spot virus (INSV), which is transmitted by insects, has severely impacted lettuce production, and in 2022 losses were reported at more than \$150 million. There are only a few insecticides that can manage thrips and there are no existing viable methods to manage the virus. ARS researchers in Salinas, California, identified important weeds and geographic locations that can serve as virus reservoirs during the winter months and can bridge the gap during the

off-season when lettuce is not grown. Results were published in the scientific journal *Plant Disease*. This has resulted in improved weed management as part of a cooperative effort by growers, industry association partners, and county-wide programs.

Soilborne oospores of the spinach downy mildew pathogen can initiate disease. Spinach downy mildew, caused by *Peronospora effusa*, is a major disease on spinach in the United States and worldwide. ARS researchers in Salinas, California, and Fort Detrick, Maryland, determined that sexual spores called oospores on spinach leaves gave rise to downy mildew on spinach when mixed with seeds at planting. The replicated experiments were performed in glass chambers in which soil was fumigated to eliminate the possibility of air or soil contamination prior to the experiments. Additional experiments also confirmed seed transmission of downy mildew disease from oospore-infested seeds. These results are used by spinach growers to address the spinach disease threat posed by soilborne and seedborne oospores.

Predicting whitefly death due to freeze events in Florida. The sweetpotato whitefly is a destructive pest on a wide variety of agricultural crops. Whiteflies are managed largely through insecticide use but they have developed tolerance or resistance to some insecticides. ARS researchers in Fort Pierce, Florida, developed a model for predicting whitefly mortality in response to freeze events in southwest Florida vegetable production fields. The model will be incorporated into a risk prediction model for managing whitefly and whitefly-transmitted viruses in vegetable production as part of an areawide pest management research effort. Other components include proximity to whitefly or whitefly-transmitted viruses and satellite identification of hosts for the insect and/or the virus. Together this novel management approach should be useful for mitigating whitefly-transmitted viruses like tomato yellow leaf curl virus and squash vein yellowing virus. This is a critical new tool for growers, crop consultants, and Extension personnel to use for predicting whitefly numbers and whitefly-transmitted virus incidence for vegetable crops. It is described in the journal *Crop Protection*.

Quickly identifying isocyanide biosynthetic genes across the fungal kingdom. Fungal secondary metabolites (SMs) enable virulence and persistence in environments and are dangerous toxins (mycotoxins) to animals that feed on infected seed or plants. Research into these compounds can help protect food safety and security. However, identifying genes associated with these compounds has been slow. ARS researchers in St. Paul, Minnesota, recently developed a new algorithm capable of finding a new class of SM called isocyanides. The researchers mapped these genes across 4,000 fungal genomes, including 144 *Fusarium* genomes, and made these results available through a web app (<https://isocyanides.fungi.wisc.edu/index.html>). This resource will enable scientists to target genes encoding isocyanides for disruption, enabling the characterization of resulting compounds and their importance in pathogenesis.

Nematode communities can reveal soil health. Nematodes are the most numerous soil invertebrate and occupy all trophic levels in the food web, from fungal and bacterial feeders to herbivores to predators. ARS scientists in Pullman, Washington, and a Fulbright Scholar from Ibn Zohn University (Agadir, Morocco) sampled potato and wheat soils across eastern Washington and Oregon, including soils that have never been cropped. Nematodes were morphologically identified in more than 30 genera and trophic levels based on mouth parts. Analysis showed that cropped soils are more disturbed and dominated by bacterial and fungal feeders, compared to native soils. These results show that nematode analysis can be used as another indication of soil health for growers, who want to know how their management systems are impacting their soils.

Laser scanning confocal microscopy Improves visualizing plant fungal interactions. Laser scanning confocal microscopy can be used to generate high-contrast 3D images for studying plant-microbe interactions. ARS researchers in Fargo, North Dakota, used wheat, barley, and sugar beet pathogens to show the utility of a staining and imaging technique that targets the fungal cell wall and DNA/RNA to visualize the plant nucleus and cytoplasm. This technique is simple and robust and can be applied to any plant-fungal interaction. Therefore, this technique is well suited to study how fungal pathogens interact with their plant host.

Grapevine host responses to virus infection. Grapevine productivity is negatively affected by infections of different viruses, including grapevine leafroll-associated virus-3 (GLRaV-3) and grapevine red blotch virus (GRBV). However, it is unclear how viruses affect host physiology. ARS researchers in Parlier, California, observed different chemical levels in grapevine leaves throughout the growing season in healthy and virus-infected vines. Higher levels of hydroxycinnamic acids (HCAs) were found in healthy grapevines. HCAs are positively associated with plant tissue maturity, so reduced levels in infected plants suggest that plant tissues in infected grapevines mature at an increased rate. These results highlight changes that viral pathogens induce in grapevine hosts, which could be used to develop biomarkers that plant diagnosticians could use to identify a potentially infected vine.

Bacterial isolates that lack protease activity result in less severe bacterial leaf streak disease. Bacterial leaf streak of wheat and barley is a global disease of growing economic concern caused by the bacterium *Xanthomonas translucens*. ARS researchers in Fargo, North Dakota, found that inoculating plants with bacterial isolates that lack protease activity resulted in reduced severity of disease symptoms compared to isolates that have protease activity. The complete genome sequencing of representative isolates showed that this protease is differentially regulated in different pathogen genetic backgrounds. Understanding the contribution of extracellular protease to virulence and the mechanism of its expression is important for developing control strategies and developing disease resistant varieties.

DNA region in wheat rust pathogen associated with virulence to wheat. Protecting the U.S. wheat crop from diseases is necessary for producing high-quality wheat to meet U.S. demands and for the United States to continue as a global leader in wheat exports. Leaf rust (Lr) caused by *Puccinia triticina* (Pt), is the most common disease of wheat in the United States and worldwide, resulting in significant yield losses on an annual basis. ARS researchers in St. Paul, Minnesota, discovered regions in DNA in Pt that are close to genes that give the fungus the ability to induce disease (virulence) in wheat. DNA regions were discovered close to virulence genes that can attack wheat cultivars with six different leaf rust resistance genes. This data can be used to find genes that allow the rust fungus to attack wheat cultivars with many different resistance genes. This information may also be used to (1) develop new rust resistance genes that are resistant to all races of Pt using advanced biotechnology methods and (2) enable next-generation pathogen surveillance techniques that track virulence factors.

The net form net blotch barley pathogen uses effector proteins to induce disease. Net form net blotch, caused by the fungal pathogen *Pyrenophora teres* f. *teres*, is a major leaf disease of barley in the United States and globally. Successful pathogens adapt to locally planted cultivars through a change and fine tuning of virulence. Unfortunately, researchers lack a firm understanding of how *P. teres* f. *teres* uses virulence (effector) genes to manipulate barley, resulting in net form net blotch disease. ARS researchers in Fargo, North Dakota, identified, validated, and functionally characterized two effector genes that target barley to cause net form net blotch disease. Understanding how this necrotrophic

pathogen is manipulating its host is critical to disease control and to helping geneticists and breeders remove the susceptibility genes targeted by these effectors.

Identifying fungi and bacteria associated with tar spot in corn. Tar spot, caused by the fungal pathogen *Phyllachora maydis*, is becoming one of the major constraints to corn production in many parts of the United States. Because resistance is not available, having biological controls that attack tar spot could help maintain yields. However, almost nothing is known about other leaf microorganisms that could serve as biological controls. ARS researchers in West Lafayette, Indiana, analyzed the leaf microbiomes of 16 lines of corn with different levels of resistance to tar spot. These analyses identified a great diversity of fungi and bacteria in corn. Analyses of the three most resistant versus the three most susceptible lines showed many species of fungi and bacteria with highly significant positive or negative associations with tar spot. Those with positive associations may be either symbionts, or parasites that feed off the tar spot causal fungus, while those that are negatively associated could be antagonists. The discovery of parasitic and antagonistic microbes may identify biocontrol organisms that could be used to mitigate losses from tar spot.

Female black widow spiders communicate through web vibrations for territorial rivalry. Female black widow spiders (*Latrodectus hesperus*) can be a phytosanitary pest in crops destined for domestic and international markets. Black widows are solitary predators of arthropods with no tolerance for intruders on the webs, suggesting that separation of black widow webs could be driven by natural territorial rivalry between females. However, female to female communication behaviors in this species remains unknown. ARS scientists in Parlier, California, demonstrated that female black widow spiders exhibit territorial rivalry, and that female to female rivalry is mediated by vibrational signals through the web. Understanding the communication behaviors used for competition in black widow spiders will facilitate developing novel methods to prevent spiders from colonizing crops. This will benefit growers and fruit packers who are concerned about black widow spiders in produce.

Crop hosts and insect vectors to help manage emerging corn virus. Maize yellow mosaic virus is an emerging virus of corn and other cereal crops that has been recently found in North America and can cause up to 30 percent yield loss. Since this virus was only recently discovered, it is not yet known which crops it can infect and which insects can transmit it. ARS researchers in Wooster, Ohio, have found that, in addition to corn, wheat, sorghum, and sugarcane, the virus can infect four additional crops: oats, barley, rye, and foxtail millet. They also identified four common weedy grass species that can be infected by the virus and might serve as a reservoir for virus when the crop hosts are not present. Furthermore, they discovered it can be transmitted by an insect known as the greenbug aphid, in addition to the two insects already known to transmit the virus. Understanding the plant hosts and insect vectors of this virus informs not only which crops may be at risk, but also how growers and extension researchers can best manage the disease using appropriate crop rotations and pest control.

Discovery of a strawberry pathogen strain that overcomes resistant plants. Fusarium wilt of strawberry, caused by *Fusarium oxysporum* f. sp. *fragariae* (Fof), is an economically damaging disease that is most effectively and economically controlled by growing a disease-resistant cultivar. The dominant gene FW1, which is found in multiple cultivars at a single locus, conveys resistance to this pathogen. FW1 was considered effective against all strains of this pathogen in California. However, an ARS scientist in Salinas, California, discovered a strain of Fof in a California fruit production field that can overcome the resistance conferred by FW1. This finding was published in the scientific journal *Environmental Microbiology*. The early detection of this new strain has enabled the strawberry industry

to launch new efforts to breed for resistance, enact a statewide surveillance program, and attempt to contain the pathogen at the site of its emergence.

Races of wheat and barley stripe rust pathogens. The wheat and barley stripe rust pathogens evolve rapidly to produce new races that can overcome resistance in currently grown varieties, and information about these races and their virulence is essential for breeding programs to use effective genes for developing new varieties with adequate and durable resistance. ARS scientists in Pullman, Washington, identified 22 races of the wheat stripe rust pathogen and 12 races of the barley stripe rust pathogen from races collected in the United States in 2022, and determined the frequencies and distributions of these races and virulence factors in various epidemic regions. The results can be used by breeders to select effective resistance genes for developing new varieties and by pathologists to select important races for screening wheat and barley germplasm for releasing new varieties with adequate and durable resistance to stripe rust.

Census for soft rot potato pathogens. There are many species of bacteria, including *Pectobacterium* and *Dickeya* species, that cause soft rot disease on potato and other crops. Soft rot diseases cause approximately one billion dollars of damage annually to potato crops worldwide and millions of dollars of damage to U.S. potato crops. ARS researchers in Ithaca, New York, and university collaborators surveyed all the major potato growing regions of the United States between 2016-2021 for bacteria responsible for soft rot diseases. The species of each soft rot bacteria was determined by analyzing the full genome sequence. Survey results, which partially are reported in two articles in *Plant Disease*, show that there are at least 11 soft rot bacterial species in the United States and that cause potato disease. *Pectobacterium carotovorum*, the most widespread, was found in 9 of the 14 states surveyed. In contrast, other pathogens are restricted to certain regions; for instance, *Dickeya dianthicola* was found only in the eastern United States. This information is essential to understanding the ecology and epidemiology of these pathogens and for informing potato seed certification programs and federal regulatory programs, which helps protect food security and livelihood of U.S. farmers.

Maize chlorotic mottle virus rarely transmitted through grain transport. Maize lethal necrosis is a devastating viral disease of corn caused by simultaneous infection of two viruses, one of which is maize chlorotic mottle virus. Maize lethal necrosis epidemics can lead to hundreds of millions of dollars in lost yields. Understanding how the virus spreads and the risks associated with seed trade is critical for preventing the spread of maize lethal necrosis. ARS researchers in Wooster, Ohio, discovered that maize chlorotic mottle virus is transmitted through seed at extremely low rates of less than 0.01 percent. The virus was found only in dead outer seed tissues that are less able to maintain viable virus particles than internal living seed tissues such as the embryo. No virus transmission was detected in seed with moisture content at or below 15 percent, which indicates proper seed drying can reduce the risk of virus spread through seeds. This information is valuable for the corn seed industry and plant health regulatory agencies for reducing the spread of maize chlorotic mottle virus through seeds, informing seed trade policies, and preventing massive economic losses associated with the introduction of maize lethal necrosis to new geographic regions.

Component 3. Plant Health Management

Release of a potato cultivar resistant to potato cyst nematodes. Potato cyst nematodes (PCN; golden nematode and pale cyst nematode) are devastating pests for U.S. potato production, which is valued at \$4.6 billion. Utilizing host resistance is the most effective and sustainable approach for PCN control. ARS

researchers in Ithaca, New York, and Cornell University collaborators developed and released ‘Bliss’, a potato cultivar that is resistant to golden nematodes. The new cultivar, which is described in the *American Journal of Potato Research*, can serve as an effective tool for golden nematode control and eradication in the United States. It can also be utilized by potato breeders as parent material in genetic crosses to develop more resistant cultivars.

Thirteen new wheat varieties with stripe rust resistance. Developing resistant varieties is the most effective, user-friendly, and environmentally sustainable approach to control stripe rust. ARS scientists in Pullman, Washington, obtained more than 16,000 wheat and barley germplasm samples from U.S. breeding programs and in 2023 used multiple field and greenhouse locations to screen the germplasm for its response to multiple races of the stem rust pathogen. They provided the data to various breeding programs for releasing new resistant varieties and to growers for selecting released resistant varieties to grow. They also used recent annual stripe rust data to collaborate with various breeding programs in releasing and registering 13 new wheat varieties with resistance to stripe rust. Growing these new varieties will reduce potential risk of stripe rust.

Cotton germplasm lines resistant to plant-parasitic nematodes. Among cotton pests, the plant-parasitic reniform and root knot nematodes are responsible for the greatest yield losses in cotton. These nematodes can also interact synergistically with root rot pathogens such as *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) to increase disease severity. Although repeated pesticide applications and crop rotations can reduce nematode damage, developing and using nematode-resistant cultivars is widely considered to be the most effective and economical approach for managing nematodes in cotton. ARS researchers in College Station, Texas, collaborated with Cotton Incorporated and academic colleagues on developing and registering eight germplasm lines of Upland cotton that are resistant to reniform nematodes and possess superior performance traits. Four of the lines also possess one or two genes that provide resistance to root-knot nematodes. These new lines will greatly help breeders develop new commercial varieties of Upland cotton that are resistant to nematodes and with resistance against Fov strains that require the presence of nematodes for infection. Results were published in the *Journal of Plant Registrations*.

Reducing food waste and stored pome fruit losses. Blue mold, which occurs during postharvest fruit storage, results in economic losses and increases food waste. It is caused by a fungus that also produces patulin, a toxin that is harmful to humans. ARS researchers in Beltsville, Maryland, and collaborators from Cornell University and Penn State University evaluated new methods to reduce blue mold spores from bin surfaces. They tested several treatments, including steam, hot water, dipping in fungicide solution, and thyme guard, and all treatments working equally well compared to untreated controls. The researchers also conclusively demonstrated that plastic bins bind fewer spores than wood, which encourages packers to replace wooden bins with plastic. Before this work was conducted, industry did not know the importance of bin sanitation, its positive impact on decay management, or practical ways to sanitize both wood and plastic storage bins. All treatments are applicable for both conventional and organic production practices, and industry is adopting bin steaming to reduce blue mold, ensure fruit quality, and reduce patulin occurrence. These results were published in the journal *Plant Disease*.

New oat lines with durable oat crown rust resistance. Producing high-quality oats in the United States requires controlling dangerous oat diseases. Oat crown rust is the most devastating disease of this crop and causes major yield losses annually, especially in organic production. The oat crown rust pathogen is highly variable and overcomes oat crown rust resistance genes when they are used singly. ARS researchers in St. Paul, Minnesota, developed two oat lines carrying a combination of three oat

resistance genes. These two new lines were much less susceptible to disease, exhibited good agronomic characteristics, and have been widely adopted by the oat breeding community; the many Material Transfer Agreements (MTAs) to date and germplasm requests are evidence of their quality. Oat breeders are using these lines as parents in their breeding programs to derive new oat cultivars with durable resistance to oat crown rust.

New bacteria group increases corn growth and produces plant hormones. Biostimulants are an important class of microbial products that stimulate plant growth and have been found to help plants grow under drought and low soil fertility conditions. ARS researchers in Peoria, Illinois, and Colombian researchers reported the discovery and characterization of *Lysinibacillus* species as biostimulants. This group of bacteria was characterized for their ability to promote plant growth in corn and to produce plant hormones in the auxin family, which are known to be a key regulator of plant growth. This study demonstrated that most of the bacteria from the genus *Lysinibacillus* could promote plant growth. Results were published in *Antonie Van Leeuwenhoek* and *Scientia Horticulturae* and expand our knowledge of how microbes interact with plants. This research benefits corn and other row crops growers in the United States by providing information that can be used to develop new products to enhance crop growth and yields.

New fungicides for stripe rust and responses of commercial wheat varieties to stripe rust and fungicide application. Stripe rust can be controlled by planting resistant crop varieties and applying fungicides when needed. To develop an integrated strategy for efficiently managing stripe rust, ARS scientists in Pullman, Washington, tested 19 fungicide treatments on susceptible varieties of winter and spring wheats to evaluate their efficacy in controlling stripe rust. They also determined yield losses caused by stripe rust and how fungicides reduced yield losses of 24 winter and 24 spring wheat varieties. The fungicide trials identified new effective fungicides and determined yield losses from stripe rust and yield increases resulting from current standard fungicide applications for individual varieties. The results can be used by chemical companies to register new fungicides and growers to determine if fungicide application will be effective for different wheat varieties and which fungicide to use for different varieties.

Protocols for rearing and handling vine mealybug facilitate pest management research. The vine mealybug is one of the most challenging pests of grapevine to control in California, with table grape growers spending up to \$500 an acre annually for control. ARS researchers in Parlier, California, developed protocols for rearing and handling vine mealybug in the laboratory. Methods for rearing vine mealybug are essential to support studies on genome sequencing, identifying biological control agents, and evaluating mealybug susceptibility to insecticides.

High temperatures during the growing season impact Pierce's disease risk. Pierce's disease of grapevine caused by *Xylella fastidiosa* has created significant economic losses to the California grape industry for decades. Colder winters in some growing regions limit pathogen survival and recurrence in perennial crops, but little is known about how climate factors affect Pierce's disease in hotter climates such as California's Central Valley. ARS scientists in Parlier, California, created a conceptual model of temperature-dependent disease severity based on summer and winter temperatures representative of California grape growing regions, and tested disease recurrence rates when infected plants experienced high summer temperatures. Results show that disease recurrence is very high under these conditions, which highlights the need for region-specific management practices that do not rely on vine recovery during the winter in areas where summer temperatures are high. This information is important for vineyard managers making decisions about when to remove infected vines and when to implement

insecticide sprays for vector control.

A nondestructive method to assess reniform nematode resistance in cotton. The reniform nematode, a microscopic worm that feeds on plant roots, is damaging to cotton. To rapidly determine if a plant has resistance to this nematode, the plant's entire root system is cut off and stained, which enables scientists to see and count the nematodes attached to it. ARS researchers in Stoneville, Mississippi, cut off the root system at either 0, 1, 2.5, or 5 cm below the soil line, and counted the number of nematodes infecting these severed portions of the root system. Resistant plants could be accurately identified while leaving up to 5 cm of roots with the shoot. They also used a combination of root retention and shoot retention treatments to evaluate the rate of plant recovery and development of bolls and seeds. Plants rapidly recovered using a treatment combination in which the top leaves and 2.5 cm roots were kept. This combination saved more than 20 days to harvest compared to some other treatment combinations. This method, which was published in the *Journal of Cotton Science*, will be useful for cotton breeders to screen their plants more effectively for resistance to reniform nematode while retaining their best materials to produce the next generation of plants.

Wheat leaf rust disease resistance gene provides protection against all races of pathogen. Protecting the U.S. wheat crop from diseases is necessary for producing high-quality wheat to meet U.S. demands and for the United States to continue as a global leader in wheat exports. Leaf rust (Lr) caused by *Puccinia triticina* (Pt) is the most common disease of wheat in the United States, resulting in significant yield losses on an annual basis. ARS researchers in St. Paul, Minnesota, discovered a new leaf rust resistance gene in wheat. This new gene gives resistance to all the current races or biotypes of Pt that occur in the United States. This gene can be used by public and private wheat breeding organizations to develop new U.S. wheat cultivars that will be highly resistant to Lr.

New genes in wheat for resistance to stripe rust. Stripe rust is best controlled through developing and growing resistant varieties. ARS scientists in Pullman, Washington, completed studies on the identification and molecular mapping of genes or quantitative trait loci (QTL) for stripe rust resistance in wheat germplasms. Through stripe rust phenotyping and molecular genotyping of bi-parental mapping populations, they identified a new gene, officially named Yr85, for resistance to stripe rust in a wheat line used to differentiate races of the wheat stripe rust pathogen. In a collaborative study, they also mapped five stable QTLs for all-stage resistance and high-temperature adult-plant resistance in wheat germplasm PI 660122 previously developed in their program. The identified genes/QTL and developed molecular markers are useful for plant pathologists to monitor races of the wheat stripe rust pathogen and wheat breeders to develop new wheat varieties with stripe rust resistance.

Proteins in tar spot pathogen suppress corn immune responses. Tar spot is caused by the fungal pathogen *Phyllachora maydis*. Plant pathogens often use proteins known as effectors to reduce host immune responses to successfully colonize a host. Though tar spot is one of the most devastating fungal diseases of corn, little is known about the effector proteins. ARS researchers at West Lafayette, Indiana, identified effector proteins that are used by *P. maydis* to suppress immune responses in corn and may have a vital role in causing tar spot disease. Eighteen candidate effector proteins were identified using an advanced *P. maydis* genome sequence and evaluated in two functional assays to determine if they interfere with well understood plant immune responses. Five of the candidates clearly showed the ability to reduce plant defense mechanisms. This work provides the first insights into how this fungal pathogen may induce disease in corn and might be used to bioengineer resistance against this devastating fungal pathogen.

Host pathway identified for nematode resistance in cotton. Hosts can respond to pathogen infection by producing compounds such as jasmonic acid (JA), which inhibit further infection. The physiological mechanisms of resistance to the root-knot nematode (RKN) in cotton are not well understood. ARS and University of Georgia researchers in Tifton, Georgia, documented that RKN infection in resistant cotton plants caused significant overexpression of multiple genes in the JA plant defense pathway, compared to the reaction in susceptible plants. This suggests a massive JA response following RKN infection in resistant plants but not in susceptible ones. These results help explain the physiological mechanisms that resistant cotton plants use to defend themselves against RKN parasitism.

Microbes vs. nematodes: Insights into biocontrol through antagonistic organisms to control root-knot nematodes. Chemical nematicides are a common approach to combat and suppress parasitic nematodes that damage crop plants, but many of these compounds are being phased out due to concerns about toxicity and environmental impacts. ARS scientists in Beltsville, Maryland, and researchers from Aligarh Muslim University (Aligarh, India) assembled comprehensive and current information on natural alternatives to chemical controls for nematodes. This work describes the ways in which these biocontrol agents, which include bacteria, fungi, their metabolites, or natural products can be exploited to control nematodes, either by directly killing them or by preventing them from multiplying in host plants. Scientists, diagnosticians, growers, action agencies, and extension agencies involved in nematode research and control will use this reference work as a guide for managing plant-parasitic nematodes.

Assessing the viability of growing low sugar and high-water crops in Florida. Beverage companies have expressed interest in growing crops that are high in water and low in sugar. One of these crops is jicama, which is newly planted in Florida. However, little is known about common plant pathogens or pests that might limit jicama production in Florida. ARS scientists in Ft. Pierce, Florida, conducted greenhouse experiments to determine if three root knot nematodes species commonly found in the region could infect jicama. Two different types of jicama were tested and the tubers were assessed for nematode infection. The researchers found that nematode galling was sporadic, and as the tuber matured, the incidence of infection was less frequent and estimated at less than 10 percent. Based on these results, root knot nematodes should not be an issue for jicama growers in Florida.

Elucidating potential of fly ash in root-knot nematode suppression on okra. Root-knot nematodes are serious pests of many economically important crops. One alternative to chemical control of root-knot nematodes is the application of soil amendments that either stimulate plant growth or inhibit nematode reproduction. ARS scientists in Beltsville, Maryland, and researchers from Aligarh Muslim University (Aligarh, India) determined that okra grown in pots with a soil amendment called fly ash showed improved soil health, growth, yield, and antioxidant properties, with fewer harmful effects of root-knot nematode compared to other treatments. The success of fly ash application for nematode control is important for scientists, growers, and extension agencies involved in nematode research and control.

Guava root-knot nematode resistance in wild watermelon. The guava root-knot nematode is an invasive plant parasite that poses a significant threat to watermelon and many other crops in the southeastern United States. ARS researchers in Charleston, South Carolina, are developing new watermelon varieties that are resistant to this pest, which will protect stakeholder yields and reduce the need to spray costly and environmentally damaging pesticides for nematode control. The researchers screened 109 wild watermelon lines for resistance to guava root-knot nematode and identified 28 resistant wild watermelon lines that will provide useful germplasm for breeding new watermelon varieties. They also collaborated with ARS researchers in Beltsville, Maryland, and Clemson University

scientists on a genome wide association study to identify wild watermelon genome regions responsible for this resistance and published their results in the journal *Plant Disease*. The data from this study will provide watermelon breeders with valuable germplasm and genetic markers to help select new watermelon varieties that are resistant to guava root-knot nematode.

Watermelon germplasm with multiple disease resistance. Watermelon is an important vegetable crop grown in 44 U.S. states, but many diseases and pests attack watermelon seedlings, plants, and fruit, which reduces yields and results in economic losses for producers. Phytophthora fruit rot and powdery mildew are two diseases that can significantly reduce watermelon yield and quality and growers generally spray expensive pesticides to manage them. ARS scientists in Charleston, South Carolina, developed a watermelon germplasm line with resistance to powdery mildew and Phytophthora fruit rot. This resistant germplasm line can be used by private and public sector plant breeders to develop watermelon varieties and breeding lines with resistance to these two diseases, which will help reduce pesticide use and enable watermelon growers to produce healthy crops. The information will be useful to growers, seed company plant breeders, University researchers, extension agents, and USDA scientists. The published research article made the cover of the April issue of the peer reviewed journal *HortScience*.

Adding desirable traits from wild relatives into cultivated peanut. Wild relatives of peanut often have beneficial characteristics that are not found in cultivated peanut. However, introducing these traits through breeding is not easy because there is often incompatibility between wild and cultivated peanuts. For the first time, ARS scientists in Dawson, Georgia, generated, validated, and characterized hybrids between two wild species. This plant material carries favorable genes to increase disease resistance and to reduce aflatoxin accumulation. Along with the development of molecular markers, this new genetic resource is ready to be used as a potential source for next-generation breeding with existing peanut cultivars to introduce favorable genes with desirable traits. It is anticipated that peanut breeders will incorporate this new germplasm into their breeding programs. The scientific manuscript was invited by the Editorial Board for publication in the peer-reviewed Journal of Visualized Experiments in 2023.

Beneficial soil microorganisms increase strawberry yields. Beneficial microorganisms can improve strawberry plant health and reduce the need to use soil fumigants. ARS researchers in Salinas, California, collaborated with a grower to test how commercially available beneficial microorganisms affect strawberry production under field conditions. Strawberry plants inoculated with beneficial microorganisms produced significantly larger strawberries compared to non-treated plants. Results from this research were published in *Plant-Environment Interaction* and are being used by growers who are interested in using beneficial microorganisms to increase strawberry yields in conventional and organic production systems.