United States Department of Agriculture Agricultural Research Service

National Program 303 PLANT DISEASES

FY 2022 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 46 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 2022 these funds were distributed across 32 competitive projects to ARS scientists and their collaborators. These projects included diseases that affect alfalfa, apple, avocado, barley, blueberry, cacao, carrot, cherry, citrus, coffee, cotton, eggplant, grasses, lettuce, oak, peanut, peppers, soybean, sugarbeet, tomato, and wheat.

During FY 2022, NP 303 scientists participated in research collaborations with scientists from Argentina, Australia, Austria, Azerbaijan, Belgium, Bolivia, Brazil, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Czech Republic, Ecuador, Egypt, Ethiopia, France, Germany, Greece, Guatemala, India, Indonesia, Ireland, Israel, Italy, Japan, Jordan, Kenya, Lithuania, Mexico, Morocco, Netherlands, New Zealand,

Nigeria, Pakistan, Panama, Peru, Philippines, Poland, Portugal, Saudi Arabia, South Africa, South Korea, Spain, Sri Lanka, Switzerland, Taiwan, Tanzania, Tunisia, Turkey, United Kingdom, and Vietnam.

The quality and impact of NP 303 research was evidenced during FY 2022 by the following:

- → 216 refereed journal articles published,
- \rightarrow 1 new invention disclosure (patent only) submitted,
- \rightarrow 18 variety and/or breeding lines released which were evaluated and/or bred for disease/pest resistance, and
- \rightarrow 1 new patent application filed.

NP 303 encompasses the following three research components in fiscal year 2022:

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 2022, 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

Precipitation changes amplify how temperature affects aphid dispersal. Rising temperatures and precipitation in some of the world's most productive agroecosystems have the potential to greatly alter insect herbivore-plant interactions in ecologically and economically significant ways. While there is growing evidence that early flights of aphids are advancing in response to warming winters, little is known about how the many dimensions of aphid phenology will respond to the combined effects of changing temperature and precipitation. ARS researchers in Urbana, Illinois, and University of Illinois and University of Georgia collaborators studied more than 500,000 of three agriculturally important aphid species collected from the U.S. Midwest Suction Trap Network between 2005 and 2019 to determine how aphid species phenology varied with seasonal temperature and precipitation. Their findings, published in the peer-reviewed journal *Ecological Applications*, determined that climate change projections with climate-aphid phenology models predicted shifts in aphid phenology by 2050 and 2080. Overall, the climate-aphid models predict that changes in climate will extend the period of crop colonization for three aphid species, possibly leading to increased damage to soybean crops and increased spread.

New class of sclerotinia fungal effector genes discovered. Plant pathogens cause disease by secreting proteins called effectors that degrade plant cells during infection, as in the case of white mold disease, which affects more than 400 different crop species across the world. ARS researchers in Pullman, Washington, discovered that the white mold fungus, in turn, produces an inhibiting effector protein that inactivates the inhibiting proteins produced by the plants to resist the disease. The researchers verified that Sclerotinia mutants with a defective effector gene caused less disease than isolates of the pathogen with the normal effector gene. This is the first example of a new class of fungal effectors that can inactivate plant defense mechanisms. The findings were published in the peer-reviewed journal *Nature Communications* and have stimulated new approaches for improving disease resistance by identifying plant effectors that cannot be inactivated by fungal effector proteins.

Improved regulatory sampling for Huanglongbing (HLB) infection. HLB is a devastating disease of citrus associated with the bacterium *Candidatus* Liberibacter asiaticus (CLas), which is efficiently transmitted by the Asian citrus psyllid, and has been found in urban settings in southern California. The first line of defense to control the spread of HLB is to eradicate infected trees. ARS researchers in Parlier, California, working with the California Department of Food and Agriculture and Central California Tristeza Eradication Agency, improved CLas detection by sampling the stems that support the flower or fruit and using real-time polymerase chain reaction for consistent isolation and detection of CLas DNA. Improving CLas detection and rapid eradication of infected trees reduces CLas spread.

Potato germplasm with novel and broad-spectrum resistance to potato cyst nematodes. The potato cyst nematode (PCN), including the golden nematode and the pale cyst nematode, are quarantine pests that pose serious threats to the multi-billion-dollar U.S. potato industry. Deploying resistant potato cultivars is the most effective and sustainable means for PCN control. ARS scientists in Ithaca, New York, screened many accessions of wild potato species in the collection at the U.S. Potato Genebank and identified several new clones that showed strong and broad-spectrum resistance against multiple populations or species of PCN. These new resistant clones are valuable for discovering novel resistance genes against potato cyst nematodes and are being introduced into U.S. potato breeding programs to accelerate breeding potatoes for durable resistance against potato cyst nematodes. Results are published in the peer-reviewed journal *American Journal of Potato Research*.

Machine learning approach to explore the bacterium that causes Pierce's disease in grape. More than 30 genome sequences of the *Candidatus* Liberobacter asiaticus (CLas) strains are now available in public databases. Genome sequences are useful for identifying genes controlling specific traits in CLas and understanding genetic relationships among different CLas strains but mining information from large sequence databases is a challenging task. ARS researchers in Parlier, California, collaborated with scientists from the National Station of Plant Epidemiology, Quarantine, and Sanitation in Queretaro, Mexico, to use a machine learning approach to analyze CLas genome sequences. A previously developed CLas strain classification system based on a single CLas virus (phage) gene was further substantiated with 12 chromosomal genes, demonstrating that machine learning is a powerful tool for genomic research in CLas.

An assay to detect viruses in dormant potato tubers. Potato is one of the most valuable food crops grown in the United States. Potatoes are produced by planting tubers from the previous year's crop, so diseases and potato pest can spread rapidly and cause significant losses. For viruses, potato seed certification relies primarily on visual field inspections during the growing season followed by post-harvest grow outs. However, new viruses emerge that do not always display symptoms, and potato seed grower are interested in obtaining seed certification results earlier, so there is a need for improving

potato tuber testing. ARS scientists in Ithaca, New York, collaborated with ARS scientists in Aberdeen, Idaho, and numerous university scientists across the United States to optimize a high -throughput diagnostic assay for viral pathogens in dormant potato tubers. This assay provides earlier detection of potato pathogens with an accuracy of 95 percent when compared to current testing methods, providing a robust alternative to current seed certification testing and enabling growers and buyers to receive data earlier. The assay can potentially be used for subsequent testing for a wide array of pathogens that are currently or potentially of interest in potato seed certification.

Best management practice recommendations for seed weevils threatening soybean production in Sub-Saharan Africa. Soybean production in sub-Saharan Africa (SSA) has increased in recent years, and several abiotic and biotic stresses, including pathogens and pests that attack seed, reduce yields. ARS researchers in Urbana, Illinois, cooperated with researchers at the Soybean Innovation Lab at the University of Illinois, who focus on improving soybean production across Africa, to report bruchid (also known as seed weevils) infested and damaged seed in seed lots in Cameroon, Malawi, Mozambique, and Rwanda. Bruchids are a destructive storage pest of legumes and monitoring the different species attacking soybean will help researchers and producers understand the potential threat of bruchids to soybean production in SSA, as well as help implement the best management practices. Results are published in the peer-reviewed journal *Plant Health Progress*.

Fungal plant pathogen database updated with new features and genomic information. *Rhizoctonia* species are devastating fungal plant pathogens causing disease on many crops, especially at the seedling stage. Accurate identification of many *Rhizoctonia* pathogens of diverse genetic composition is essential for the selection of appropriate disease control tools, because morphologically similar *Rhizoctonia* species complex isolates can vary in sensitivity to chemical or biological control measures. ARS scientists in Beltsville, Maryland, and scientists from Saudi Arabia collaborated on developing six additional annotated *R. solani* genomes for the database *RsolaniDB* for a total of 18 genomes. *RsolaniDB* was also improved to make it more resourceful for pangenomic comparative investigations and for developing methods for identifying different isolates within the *R. solani* species complex. This database, which has been cited in the peer-reviewed journal *Frontiers in Microbiology*, is a tremendous resource for plant pathologists, mycologists, and molecular biologists devising specific molecular detection methods for members of this morphologically similar but genetically different complex of economically important plant pathogens.

The whole genome sequence of a *Xylella fastidiosa* (*Xf*) strain causing phony peach disease. Phony peach disease (PPD) is a potentially destructive disease that has been in the southeast United States for more than a hundred years and is caused by the *Xf* bacterium. Little is known about the biology and genomics of *Xf* strains causing PPD. ARS researchers in Parlier, California, collaborated with ARS scientists in Byron, Georgia, and University of Georgia scientists to collect bacterial samples from Georgia and Alabama and sequence the genome of a selected strain using a metagenomic approach. The genome sequence provides a valuable resource for scientists studying the biology and taxonomy of *Xf*, and the information can be useful for PPD management and for regulatory agencies.

New diagnostic method to detect a spinach pathogen in leaves. Downy mildew disease of spinach, caused by the plant pathogenic microorganism *Peronospora effusa*, is the major disease constraint on spinach in the United States and worldwide. ARS researchers in Salinas, California, led the effort to develop a species-specific detection system that can be used in the field. The system is DNA-based and can be deployed from a pickup truck, detecting the pathogen within 3 to 4 hours. This new early detection technology will help growers target fungicide applications more effectively prior to symptom

development, and thereby help to prevent downy mildew epidemics. Since nearly 45 percent of U.S. spinach is organic, the technology is especially helpful to organic growers who may choose to harvest organic crops earlier and avoid symptom development that renders their product unmarketable. Results are published in the peer-reviewed journal *Plant Disease*.

Development of a rapid diagnostic test for tomato apical stunt viroid. Tomato apical stunt viroid (TASVd), a small nucleic acid plant pathogen, is a serious threat to tomato production worldwide; infection leads to reduced plant vigor, small and deformed fruit, and yield loss. TASVd is seed-transmitted and is easily transmitted mechanically from plant to plant once introduced, and seed treatments do not control disease transmission. Tomato seed imports into the United States require a phytosanitary certificate stating the seed are free from quarantine pospiviroids, including TASVd. Current detection methods require costly equipment and specific training. ARS scientists in Beltsville, Maryland, developed rapid, specific, sensitive, and user-friendly tests for TASVd detection in leaf and seed tissues that can be employed in the laboratory and in the field for on-site diagnosis. Results are published in the peer-reviewed journal *Journal of Virological Methods*. These tests will be useful to scientists, growers, the industry, and regulatory agencies, who are developing methods and protocols to control viroid diseases.

Peanut smut cultivation. Peanut smut, caused by a fungal pathogen, is a grave disease of peanuts that threatens peanut production worldwide. Although currently not present in the United States, it is a potential threat to the U.S. peanut industry because there are no known commercial resistant cultivars. Developing resistant peanut cultivars requires assessing variations in smut strains, which requires lab culturing of large quantities of the pathogen. Until now there were no published papers on peanut smut cultivation, and existing reports were incomplete and unreliable. Scientists at the ARS-associated laboratory in Hurlington, Argentina, developed a reliable cultivation method for smut using standard culture media supplemented with peanut peg extracts. This is a major step toward being able to study this pathogen and manage its impact and spread.

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

Pathogen proteins that disable plant immune systems. During the infection process, plant pathogens inject proteins known as 'effectors' into the plant host cell to disable the plant immune system, promoting pathogen growth and disease and consequently reducing crop yields. Tar spot of corn is caused by the fungal pathogen *Phyllachora maydis* and can significantly reduce overall corn yields. The disease can significantly affect farmer profits and more information is needed about how this fungal pathogen uses its proteins to infect corn leaves. ARS researchers in West Lafayette, Indiana, observed how *P. maydis* interferes with and manipulates host immune responses and found that this fungus injects effector proteins into host cells, where they specifically target plant organelles such as the nucleus and chloroplast. This work provides valuable insights into the host processes potentially manipulated by this fungal pathogen. A manuscript describing these results was recently published in the peer-reviewed journal *Phytopathology*.

Glassy-winged sharpshooters avoid plants treated with systemic insecticide. The glassy-winged sharpshooter (GWSS) is a vector of the bacterial pathogen *Xylella fastidiosa*, which causes numerous economically important plant diseases, including Pierce's disease of grapevine. The neonicotinoid insecticide imidacloprid is used to suppress GWSS populations and can be applied via irrigation systems; then it is taken up by the plant roots and circulated through the plant via the vascular system. ARS

researchers in Parlier, California, determined that GWSS cease feeding on imidacloprid-treated plants and avoid them, suggesting that application of soil-applied imidacloprid is more likely to push GWSS out of treated habitats rather than kill them. Results published in the peer-reviewed journal *Plant Disease* can be used by growers and regulators to ensure that the most effective means of controlling GWSS are implemented.

Improved weed management to combat an insect-transmitted virus affecting lettuce. Lettuce production in California's Salinas Valley accounts for more than 50 percent of total annual U.S. production, but since 2018, impatiens necrotic spot virus (INSV), which is transmitted by thrips, has severely impacted lettuce production. In 2021, more than 750 fields reported disease incidence, and crop losses exceeded \$100 million. Results from a 2020 survey indicated that higher pest management costs associated with efforts to manage the insect were estimated to increase total farming costs 10-15 percent. There are only a limited number of insecticides that can control thrips and there are no existing methods for INSV management. ARS researchers in Salinas, California, identified weeds and locations that serve as virus reservoirs during the winter months when lettuce is not grown. These findings are being used by growers for improved weed management and by the California Department of Transportation, which is managing weeds along major highways throughout the Salinas Valley.

Durum wheat susceptibility to the barley pathogen that causes spot form net blotch. Spot form net blotch (SFNB) of barley is caused by the fungus *Pyrenophora teres f. maculate* and is an emerging disease in major barley growing regions of the world. It is a particularly important problem in U.S. barley production regions. ARS researchers in Fargo, North Dakota, found that *P. teres f. maculata* is also highly virulent on popular local durum wheat cultivars, information about this pathogen that was previously unknown, and used a durum mapping population to identify the genetic location of a major gene contributing disease susceptibility in durum wheat. Confirmation that this pathogen has emerged as a durum wheat pathogen is critical for extension pathologists making recommendations to growers and breeders who will need to select for SFNB resistance in durum wheat. These results were published in the peer-reviewed journal *BMC Genomics*.

How plant water deficit affects development of Pierce's disease of grapevine. Pierce's disease is a lethal bacterial disease of grapevines caused by *Xylella fastidiosa* (*Xf*), which can rapidly spread within vineyards, requiring complete replacement in a matter of years. Water availability is increasingly challenging for grape growers in California, and one solution is to periodically reduce irrigation to save water. However, the effects of deficit-regulated irrigation on the development of Pierce's disease are unclear. ARS scientists in Parlier, California, determined that a month-long period of water stress prior to inoculation of *Xf* greatly increased Pierce's disease development and plant mortality. These findings suggest that the practice of deficit irrigation in grapevine orchards where Pierce's disease is endemic requires increasing other management techniques to control the spread of *Xf*.

New guidelines for naming phytoplasma species. Phytoplasmas are insect-transmitted bacterial plant pathogens that inhabit the plant host phloem and cause devastating yield losses. The first guidelines for separating phytoplasma species were proposed by an international working team of experts in 2004, which has served nearly 20 years. In recent years, with the rapid development of genomics and sequencing technology, important progress in bacterial taxonomy was achieved. To embrace these new developments and to incorporate "whole genome" concept to phytoplasma taxonomy, an ARS scientist in Beltsville, Maryland, in collaboration with worldwide scientists, proposed revised guidelines. This accomplishment will provide a new standard for phytoplasma researchers to name new species.

Machine learning approaches identify root microbial community members associated with Prunus replant disease. Prunus replant disease (PRD) is a serious but poorly understood soilborne disease complex that suppresses tree development, efficient water and nutrient use, and crop yield in more than 10,000 replanted almond orchards every year. Integrated PRD management requires a better understanding of factors that drive its occurrence. Machine learning approaches focusing exclusively on root microbial populations resulted in identifying 26 bacteria, 2 oomycetes, and 2 fungi as top predictors of PRD induction. *Streptomyces scabiei, Steroidobacter denitrificans, Streptomyces bolbili*, and *Pythium mamillatum* were relatively abundant (5-43 percent) among the top predictors. The findings will guide future targeted testing of microbial taxa for PRD induction and suppression in roots.

Improved DNA extraction from soil for enhanced disease risk assessment. The ability to quantify soilborne fungal plant pathogens in a field prior to planting would help growers assess risk and make management decisions to reduce losses. However, for many types of fungi, techniques to facilitate this are not available. An ARS scientist in Salinas, California, developed molecular assays capable of quantifying several of the important pathogens of strawberry and vegetable crops often grown in rotation, as well as an improved soil DNA extraction procedure that results in purified DNA from larger amounts of soil than previously used. These accomplishments have improved the ability to accurately quantify lower levels of the pathogens and thereby provide more accurate data to growers.

Genes involved during infection process of downy mildew. Downy mildew infects susceptible basil cultivars every year in the United States and can cause significant losses in yield and crop quality. The pathogen responsible for downy mildew disease on basil has not been well studied at the molecular level, primarily because the pathogen will not grow on artificial growth medium, but only on living plants. ARS researchers in Peoria, Illinois, collaborated with scientists from Israel and the University of Hawaii to identify the genes in basil plants that are turned on during the course of the disease. In addition, genes were identified that the pathogen uses during the infection process. Knowing about the nutrients the pathogen is utilizing will enable researchers to better develop methods to cultivate it outside the plant. These results contribute important knowledge of how downy mildew infects basil and could aid in developing more effective measures for reducing the severity of the disease.

Component 3. Plant Health Management

New genes for stripe rust resistance and new wheat germplasm. Stripe rust is best controlled through developing and growing resistant varieties. From 2017 to 2021, ARS scientists in Pullman, Washington, published 43 papers from their studies and collaborative studies on the identification and molecular mapping of genes or quantitative trait loci (QTL) for stripe rust resistance in wheat and barley. They identified and mapped hundreds of genes or QTL (including at least 100 new genes) for stripe rust resistance in 22 bi-parental populations and 18 genome wide association study panels, including more than 5,000 wheat entries and 20 (including five new) genes or QTL for stripe rust resistance from two bi-parental populations and 300 barley entries. They developed and registered 29 new wheat lines, including 15 lines that each carry two genes on the same chromosome arms. The identified and developed stripe rust resistant germplasm lines, effective genes, and linked molecular markers have been used by many breeding programs for developing new wheat and barley varieties.

Survey of potential impact of nematodes on organic soybean production. Plant-parasitic nematodes feed on soybean roots, which results in yield losses. Surveys of nematodes in certified organic soybean fields have been infrequent compared to surveys in non-organic soybean fields. ARS researchers in

Urbana, Illinois and University of Illinois researchers conducted a nematode survey in certified organic soybean fields in northern and central Illinois to determine the frequency and population densities of plant-parasitic and free-living nematodes. Fields surveyed included both long-term (5+ years) rotation, and short-term (3-year) rotation with soybean. Common plant-parasitic nematode taxa included spiral, lesion, cyst, stunt, dagger, and pin nematodes. Among the taxa, spiral, lesion, and dagger nematodes were above previously reported damage threshold levels in some fields. Cyst nematode population densities were significantly higher in fields under short-term rather than long-term rotation with soybean. This knowledge, which was published in the peer-reviewed journal *Plant Health Progress*, will allow organic growers to make intelligent control decisions to mitigate yield loss to nematode damage.

Broad resistance to Phytophthora fruit rot in USDA developed watermelon germplasm. Watermelon is an important crop grown in 44 U.S. states. Many different pests and diseases attack watermelon plants and cause extensive damage. In recent years, an old, but re-emerging disease called Phytophthora fruit rot has been causing serious problems in watermelon production in many states. Weather conditions for fruit rot development are always prevalent in the U.S. Southeast and the pathogen can infect the fruits at any stage. ARS researchers in in Charleston, South Carolina, developed and released Phytophthora fruit rot resistant germplasm lines for use by plant breeders. They demonstrated that the germplasm lines were resistant to 20 isolates of the plant pathogen collected from nine different states and different crops from across the United States. These resistant germplasm lines can be used to develop resistant watermelon cultivars for managing the disease, thus helping reduce pesticide use. These fruit rot resistant watermelons accessions and the information will be useful for public and private plant breeders for incorporating fruit rot resistance in watermelon cultivars.

Genes involved in plant defense in resistant durum wheat. The plant disease Fusarium head blight (FHB) is an important constraint to profitable production of durum wheat in the upper U.S. Midwest. Currently, planted durum cultivars lack consistently high levels of resistance to the disease. To identify new genes for disease resistance, ARS researchers in St. Paul, Minnesota, studied differences in the genes expressed by disease susceptible lines and a newly developed M4 line that has moderately high FHB resistance. During infection, the resistant M4 line activated many categories of genes associated with an active plant disease defense response. These genes are being further characterized to better define the mechanism of resistance and assist in breeding better cultivars. These new native sources of FHB resistance in durum wheat with the gene markers will be a great boon to the effort in breeding for resistant cultivars and the growers in the Upper Great Plains of the United States.

Sequencing of wheat stem rust resistance gene for different stem rust strains. Wheat stem rust caused by the fungal pathogen *Puccinia graminis* f. sp. *tritici* is an emerging threat to wheat production because of the detection of virulent strains such as Ug99. Effective wheat resistance genes, such as Sr9, are needed to develop wheat varieties with durable stem rust resistance. ARS researchers in St. Paul, Minnesota, identified six variants in the Sr9 wheat stem rust resistance gene in collaboration with scientists from CSIRO and the University of Sydney. Gene modification techniques were used to confirm that four of the variants had changes in a specific type of immune receptor that conferred resistance to a unique spectrum of wheat stem rust pathogen isolates. The identification of these alleles will enable them to be combined with other effective genes in wheat breeding, while the high similarity between these proteins will greatly assist in understanding the structure and function for the design of new resistance specificities so that plant pathologists and breeders can develop new resistance to protect U.S. wheat from stem rust losses.

New maize germplasm with elite resistance to maize lethal necrosis disease. Maize lethal necrosis (MLN) of maize is caused by a complex of several viruses and was first detected in the United States in the 1970s. The disease has since spread globally, recently causing devastating yield losses in East Africa, Southeast Asia, and South America. ARS researchers in Wooster, Ohio, developed a maize population with elite resistance to MLN and its causal viruses, maize chlorotic mottle virus (MCMV) and different potyviruses. The population, named OhMCMV-1, was made from five parental lines with elite MLN resistance. Lines in this population are significantly more resistant to MLN, MCMV, and potyviruses than the parental lines. The strong virus resistance in this population will be used by breeders to develop new, elite breeding lines, hybrids, and cultivars to mitigate the impact of MLN worldwide. OhMCMV-1 was publicly released and deposited in the National Plant Germplasm System, where it is available for research and development.

Management of soilborne pathogens, pests, and weeds with natural products. Soil fumigants, especially methyl bromide, are commonly used to control soilborne pathogens, pests, and weeds. However, methyl bromide use is restricted because of its ozone depleting negative impact on the environment. Alternatives to methyl bromide for soil fumigation are urgently needed. ARS scientists in Fort Pierce, Florida, investigated three natural products for their potential to manage soilborne pathogens and pests and weeds that severely limit plant health and crop yield. They demonstrated that Allylisothiocyanates (AITC), an organosulfur compound naturally found in mustard and other members of the Brassicaceae family, was a successful bactericide, nematicide, and fungicide, but provided very poor weed control; however, they showed that AITC could be combined with herbicides to successfully manage plant pathogenic microbes, plant parasitic nematodes, and weeds. The researchers also demonstrated that chitin, which is found in the shell of crustaceans, negatively impacted the incidence of root-knot nematodes in cut flowers. In addition, the scientists demonstrated that dimethyl disulfide (DMDS), another organic compound, was successful as a soil fumigant for managing soilborne pests and pathogens. DMDS has a strong odor that has limited its adoption by growers, but ARS scientists showed that DMDS paired with oak residue biochar reduced the odor of DMDS and maintained its nematicide activity. This research provides growers, especially organic growers, more tools for combating soilborne pathogens, pests, and weeds.

Cell phone app to track tree fruit pests. Tree fruit diseases and pests cause food loss during storage and in the field. ARS researchers in Beltsville, Maryland, in collaboration with Partnerships in Data Innovation and numerous university scientists, developed, tested, and launched a new cell phone app and dashboard to record, map, and track diseases of tree fruits. The publicly available tool PATHMAP can help inform stakeholders and customers about regional outbreaks and hosts to help manage pests. This tool will assist in disease diagnosis and pest management to reduce their spread locally and globally as fruit are exported to other countries.

Development of a modified powdery mildew risk index for hops. A strain of the hop powdery mildew fungus has emerged in the western United States. In 2012, the strain was aggressive on the cultivar 'Cascade', the most widely grown cultivar at the time, which previously was not severely affected by the disease. ARS researchers in Corvallis, Oregon, characterized how this strain of the fungus and Cascade respond to high temperature, and used this knowledge to develop a revised disease risk index to better inform growers. A beta version of a web app and an automated email delivery system of the powdery mildew risk index was developed in partnership with university collaborators for validation and dissemination to stakeholders. In field testing, use of the risk index reduced the number of fungicide applications required by as much as one-third compared to standard practices in certain years.

Rootstocks for managing nematodes in Washington wine grapes. Plant-parasitic nematodes are pests that limit production in most grape growing regions of the world. In many places, rootstocks are used to reduce the impact of nematodes on grape production, but this is not a common management practice in Washington, the second largest producer of U.S. wine. ARS researchers in Corvallis, Oregon, showed that rootstocks are poor hosts for the most common plant-parasitic nematode in the region. This research provides Washington wine grape growers with a sustainable means to manage plant-parasitic nematodes that they are now seriously considering for adoption.

Alternative soil amendment-based strategies to control apple replant disease (ARD). ARD occurs when multiple soilborne pathogens diminish the productivity of established trees and slow the growth of new plantings. Control strategies using commercial ARD fumigation are becoming less viable due to increasing regulatory/environmental constraints. ARS researchers in Wenatchee, Washington, determined that soil amendments and anaerobic soil disinfestation (ASD) can harness the potential of commercially available apple rootstock genotypes and their root-associated microbiomes to tolerate and/or suppress replant pathogens. This research helped to define effective amendment rates and the best ASD carbon source for integrating organic inputs with commercially available apple rootstock genotypes to control soilborne pathogens. Currently, there are limitations to widespread adoption at the commercial field scale due primarily to the initial cost compared to fumigation. However, this research, which was published in the peer-reviewed journal *Frontiers in Microbiology*, highlights the long-term and sustainable benefits to the grower.

Comprehensive research to manage and reduce the spread of an emerging virus on tomato. Tomato is one of the most important vegetable crops in the world, and the United States is the third largest tomato producer (more than \$2 billion farm gate value). Tomato is especially susceptible to many viral diseases, which are difficult to manage. An emerging virus, tomato brown rugose fruit virus (ToBRFV), has caused serious disease epidemics around the world, especially in U.S. greenhouse tomato production. ARS researchers in Charleston, South Carolina, made the first report of a ToBRFV outbreak in the United States in 2019. Following that report, USDA-APHIS issued a Federal Order to quarantine and control this pest. As a result, the researchers conducted a comprehensive evaluation on two key biological properties for ToBRFV, host range and disease resistance breaking. They also developed a sensitive and species-specific detection method that could be useful for seed health testing. To help reduce spread, the researchers identified several disinfectants effective against ToBRFV on tomato, which are now used by U.S. greenhouse tomato growers. Identification of several common disinfectants with similar efficacies against two related virus species on different plants suggest their broader effects against other viruses.

Optimization of anaerobic soil disinfestation. Anaerobic soil disinfestation (ASD) is a biologically based preplant technique that is shown to be as effective as methyl bromide for managing weeds and soilborne plant pests and pathogens. ASD in Florida consists of amending the soils with a feed-grade molasses and then tarping and watering the soil. ARS scientists in Fort Pierce, Florida, surveyed growers to discover what could increase grower adoption of ASD. One of growers' top concerns was the availability and cost of feed-grade molasses. The ARS researchers collected and assessed waste products, such as algae, common weeds, cover crops, and food industry byproducts, to determine if these products could be used as alternatives to molasses. The researchers demonstrated that of the materials tested, various cover crops, including cowpea and sunn hemp, can be successfully incorporated to reduce the amount of molasses required by providing supplemental carbon and nitrogen. Furthermore, ARS scientists developed a microbial slurry from the components of ASD, which, when added to molasses, created an anaerobic environment that lasted longer than traditional ASD

applications. The overall impact of this research could reduce ASD application costs and encourage grower adoption.

Bioplastic used to deliver agricultural chemicals and biocontrol agents. Biocontrol has emerged as a very important strategy for mitigating aflatoxin contamination in vital crops such as corn, peanuts, cottonseed, and tree nuts. In the initial phases of developing the technology, effective biocontrol strains were developed, but their application was inefficient and wasteful because they had to be applied to the soil before it was known if aflatoxin would be a problem that season. ARS researchers in Stoneville, Mississippi, in collaboration with the University of Bologna and the University of Minnesota, showed that bioplastic made from cornstarch, which is biodegradable and environmentally friendly, has a variety of valuable properties that enable it to be used for the delivery of pesticides and biological control agents. They demonstrated that the inert bioplastic is stable and compatible with most agrochemicals and it pass through a spray head and stick to leaf surfaces more efficiently. In addition, when combined with biocontrol microorganisms, it provides nutrition to the microbes for its survival and proliferation on the leaf surfaces. In addition, suspensions of agrochemicals and biocontrol microorganisms with the bioplastic will dry to form a hard and durable coating on seeds. This research has had major impacts in agricultural biotechnology research nationally and internationally and is currently being evaluated by a major agricultural chemical company for commercial development.

Quantitative trait loci (QTL) for durable leaf rust resistance in wheat. Leaf rust caused by *Puccinia triticina* is the most common disease of wheat in the United States and worldwide, resulting in significant yield losses annually. The leaf rust fungus is highly variable for virulence, with many different races occurring each year in the United States. As a result, obtaining durable leaf rust resistance in wheat cultivars has been difficult to achieve. ARS researchers in St. Paul, Minnesota, determined the chromosome location of genes in the soft red winter wheat breeding line CI13227, which has had long lasting resistance to leaf rust. A population of wheat derived from crossbreeding CI13227 with a susceptible spring wheat, *Thatcher*, were genotyped with DNA markers and tested for leaf rust resistance data indicated that the chromosomal locations (QTL) for leaf rust resistance in CI13227 were present on three chromosomes. DNA based markers were developed that will enable wheat breeders and plant pathologists to select germplasm that carries these resistance genes.

Determining the location of a leaf rust resistance gene in wheat. Leaf rust caused by *Puccinia triticina* is the most common disease of wheat in the United States and worldwide, resulting in significant yield losses on an annual basis. The leaf rust fungus is highly variable for virulence, with many different races occurring each year in the United States. As a result, many of the previously characterized and mapped leaf rust resistance genes no longer provide any effective resistance to the current *P. triticina* population in the United States. ARS researchers in St. Paul, Minnesota, determined that a potentially new leaf rust resistance gene was present on chromosome 1DS. This gene provides resistance to the current *P. triticina* population in the United States. Nine DNA markers were used to map this gene, which can also be used by wheat breeders and plant pathologists to select wheat germplasm with the gene.