United States Department of Agriculture
Agricultural Research Service

National Program 303 PLANT DISEASES

FY 2021 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 47 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 2021 these funds were distributed across 30 competitive projects to ARS scientists and their collaborators. These projects included diseases that affect alfalfa, avocado, cacao, coffee, eggplant, mango, oak, peanut, peppers, potatoes, soybean, sweet potatoes, tomato, watermelon, and wheat.

The United Nations had declared 2020 and 2021 as the International Years of Plant Health (IYPH). This declaration recognizes the importance of plant health and management of plant diseases to maintain food security throughout the world. NP 303 research projects contributed to the goal of the IYPH to raise global awareness on how protecting plant health can help end hunger, reduce poverty, protect the environment, and boost economic development most effectively through collaborative research. Many of the NP 303 research projects include significant domestic and international collaborations including government, industry, and academia. These collaborations provide opportunities to leverage funding and scientific expertise for USDA-ARS research and accelerate dissemination of ARS research results, thus enhancing the impact of ARS research programs. During FY 2021, NP 303 scientists participated in research collaborations with scientists from Argentina, Australia, Austria, Azerbaijan, Belgium, Brazil, Bulgaria, Cambodia, Canada, Chile, China, Colombia, Costa Rica, Czech Republic, Ecuador, Egypt, Ethiopia, Finland, France, Germany, Greece, India, Ireland, Israel, Italy, Jamaica, Japan, Jordan, Kenya, Lithuania, Mexico, Morocco, Netherlands, New Zealand, Nigeria, Peru, Philippines, Portugal, Russia, 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 2021 by the following:

- → 270 refereed journal articles published,
- → 2 new invention disclosures submitted,
- → 25 variety and/or breeding lines released which were evaluated and/or bred for disease/pest resistance,
- → 2 new patent applications filed, and
- → 1 new patent issued.

After an external retrospective review panel reviewed all facets of NP 303, a new 5-year Action Plan was approved in 2021 to guide NP 303 research objectives for 2022 to 2026. This new Action Plan fits under the general guidance of the USDA Science Blueprint and supports the USDA ARS Strategic Plan. This new plan continues to build from the existing 2017-2021 Action Plan to improve and expand our knowledge of existing and emerging plant diseases and develop effective and sustainable disease management strategies.

NP 303 encompasses the following three components in fiscal year 2021:

Component 1. Etiology, Identification, Genomics and Systematics

Component 2. Biology, Ecology, and Genetics of Plant Pathogens and Plant-Associated Microbes

Component 3. Plant Health Management

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 2021, this program produced many important discoveries and advances. Select accomplishments are described below, grouped by program component.

Component 1. Etiology, Identification, Genomics and Systematics

New viruses passed on by whiteflies threaten cucurbit crops in California's Central Valley. In the U.S. Southwestern low desert, two yellowing viruses are known to impact summer and fall cucurbit production. These two viruses had not been previously known to infect plants in the Central Valley of California, where more than half of U.S. cantaloupe production occurs. During the fall of 2020, ARS scientists in Salinas, California, identified both viruses from melon plants in Fresno County, California, using a virus detection system developed by the ARS laboratory. These first reports were published in the journal *Plant Disease.* The viruses have the potential to cause severe losses, and it is important for the cucurbit industry that continued monitoring occur to determine prevalence and to develop strategies to reduce the impact of the virus.

Identification of novel variants to the sudden oak death pathogen. Sudden oak death is caused by the water mold *Phytophthora ramorum* and continues to invade U.S. forests. To control outbreaks in Oregon forests, forest managers need to understand how the pathogen is introduced and where it comes from. ARS scientists in Corvallis, Oregon, detected novel variants of the sudden oak death pathogen using genetic analysis. This analysis identified novel introductions of two clonal lineages into U.S. forests. This information provides insights for regulatory agencies and forest managers to understand pathways of introduction of the sudden oak death pathogen.

Two for one: New technology detects two citrus pathogens in one test. Citrus greening is managed in California by intensive surveys and immediate eradication. Detecting citrus greening is complicated by the presence of citrus stubborn disease, which is caused by a different pathogen than the citrus greening pathogen. However, trees infected with either pathogen exhibit symptoms that are similar, resulting in misidentification and complicating citrus greening control and eradication efforts. ARS researchers in Parlier, California, developed two different PCR tests that can rapidly differentiate between the two pathogens. These methods, published in the online journal *PLoS One*, provide clear quantitation of the pathogen(s), which is critical when there are only low concentrations of target DNA, and is critically important in regulatory programs involving mandatory eradication.

DNA sequence resources for bacterial plant pathogens of economic importance. *Xylella taiwanensis* (Xt) causes pear leaf scorch disease in Taiwan and *X. fastidiosa* (Xf) is an important bacterial pathogen that causes plum leaf scald disease (PLSD) and phony peach disease in the southeastern United States. ARS researchers in Parlier, California, collaborated with scientists in Taiwan and Georgia to sequence a PLSD strain and issued the first report of a whole genome sequence of a Xf strain from plum. A small plasmid was found in Xt with a genetic makeup that is 65 percent similar to plasmids from Xf and other bacterial species. This is the first evidence suggesting that exchange of genetic material between these bacterial species might occur. Plasmids are associated with bacterial virulence and environmental adaptation, and the results provide clues to eventual control of these devastating diseases.

Training dogs to detect early infection of plants by pathogens. Early detection and rapid response is key to the mitigation of all plant diseases. Representative samples for testing can be difficult to collect, especially early in the growing season or epidemic when pathogens are at their lowest levels. This same caveat applies at the individual plant scale when a pathogen is incompletely distributed in its plant host, particularly with large and/or perennial host plants. Both these sampling scenarios can lead to false negative test results. ARS researchers in Fort Pierce, Florida, and industry cooperators demonstrated that canine detection of plant pathogens is a novel approach that is rapid, sensitive, and reliable for bacterial and viral plant pathogen detection. Since canines holistically and non-destructively assay the entire plant, sampling issues can be avoided at both agroecosystem and individual plant scales while maintaining testing accuracy. Real-time canine detection generates immediate results that can be translated into immediate corrective actions by growers.

New technique for sampling nursery water can rapidly detect pathogen causing Sudden Oak Death. *Phytophthora ramorum* (*P. ramorum*), a fungal-like pathogen that causes sudden oak death, continues to threaten U.S. forest ecosystems and the nursery industry. Rapid methods are needed for recovery and detection of pathogen spores from water sources, as current methods can take 8-10 days to complete. Working at the USDA-funded National Ornamental Research Site at Dominican University of California, ARS scientists in Fort Detrick, Maryland, are developing methods for rapid recovery and detection of *P. ramorum* from nursery irrigation water sources. Results with spiked samples taken from a mock nursery irrigation pond indicated that the pathogen can be detected in 24 hours or less in nursery water samples. These findings have significantly reduced the time for diagnostic labs to report results to regulatory agencies. Reducing the time frame from sampling to detection will allow regulatory agencies to quickly identify infested nursery sites and reduce the risk of shipping *P. ramorum*-infected material out of quarantine zones.

Population diversity, dynamics, and differentiation of the wheat stripe rust pathogen in the United States. Molecular characterization of the stripe rust pathogen population is important for understanding the pathogen variation and epidemiology of the disease. ARS scientists in Pullman, Washington, published a paper on molecular characterization of U.S. wheat stripe rust collections from 2010 to 2017. Using a set of simple sequence repeat (SSR) markers, they identified 1,454 genotypes from 2,247 isolates, and clustered them into three major molecular groups (MGs) and 10 sub-MGs. Researchers determined that western populations had more genotypes and higher diversities than the eastern populations. Pathogen population varied from year to year and from region to region, and gene flow was stronger among the regional populations in the east than in the west. Scientists identified a new MG and five sub-MGs that have been introduced or developed in the United States since 2011, identified a moderate but significant correlation of the molecular data with the virulence data, and determined that the U.S. wheat stripe rust populations are asexually reproduced. This study improves the understanding the mechanisms of the pathogen evolution, and the results will be useful for managing wheat stripe rust.

Viruses detected in ARS cherry collection. Three recently described viruses—cherry virus F (CVF), prunus virus F (PVF), and cherry virus T (CVT)—were detected in sour cherry accessions from the ARS sour cherry germplasm repository in Geneva, New York. This is the first report of any of these viruses in the germplasm collection, and the first report of CVT in the United States. The complete genome sequence of this CVT isolate was determined and genome sequences of the three viruses were used to develop reliable detection methods that can be used to screen cherry genetic material. An additional 100 sour cherry accessions from the gene bank were tested for the three viruses. Both CVF and PVF were frequently detected, but CVT infections were rare with an incident rate of only 1 percent. This information helps develop better viral control measures for the germplasm repository, along with others who breed, propagate, and use cherry germplasm.

Development of training manual and virtual workshops for grain inspections. Dwarf bunt of grain, caused by the fungal pathogen, *Tilletia controversa Kuhn* (TCK), is of quarantine significance. If certain levels of TCK are found in a shipment of wheat, the shipment may be refused, so it is important to inspect grain samples prior to shipment. Currently, trained personnel conduct visual inspections and must know how to identify TCK, which can resemble other fungal spores that are not of quarantine significance. An ARS scientist in Frederick, Maryland, developed a training manual for quantitating teliospores of TCK in grain samples and conducted virtual workshops on the Dwarf bunt testing procedure that was attended by individuals from USDA, universities, U.S. Wheat Associates, Wheat Marketing Center, Cargill, and several private testing laboratories. The Federal Grain Inspection Service (FGIS) adopted the training manual as the official USDA protocol. Training inspectors is critical to the proper identification of this pathogen and assurance of quality control for trade purposes.

Scalable molecular markers for hop powdery mildew genotyping. Managing emerging and endemic plant diseases often is limited by not knowing which pathogen strains are present and not knowing traits associated with the strain’s geographic origin, fungicide resistance, and host range. This is the case with the hop powdery mildew fungus, which exists in unique populations in different parts of the United States and world. ARS researchers in Corvallis, Oregon, and Geneva, New York, and university collaborators identified a set of molecular markers that can predict whether a given strain of the hop powdery mildew fungus is exotic or endemic to the western United States; they can also be used to assess virulence. The markers were successfully adapted for a high throughput genotyping system that can simultaneously evaluate hundreds of markers on hundreds of samples. This technology will be foundational for rapidly responding to new disease outbreaks and tailoring management to match the specific strain of the fungus present.

Oat crown rust virulence in the United States has increased dramatically. Crown rust, caused by the fungus *Puccinia coronata f. sp. avenae* (Pca), is an important disease of oat in the United States. Many new races of crown rust appear every year in the United States. The new races often overcome the crown rust resistance genes in oat cultivars. ARS scientists in St. Paul, Minnesota, determined that the average crown rust isolate collected from the oat growing regions of the United States gained the ability to overcome nine crown rust resistance genes in oat between 1993 and 2017. The average isolate collected during the 2017 growing season can overcome 17 of the 26 oat resistance genes tested. This recent Pca population is capable of overcoming many more oat crown rust resistance genes than the populations from previous years. This result strongly undermines the strategy of how crown rust resistance genes are used in many oat breeding programs and suggests that any combination of previously deployed resistance genes will be quickly overcome by the Pca population.

New races of the wheat and barley stripe rust pathogens identified. Wheat and barley stripe rust pathogens evolve rapidly to produce new races that can overcome resistance in currently grown varieties. More information about races and their virulence factors is essential for breeding programs to use effective genes for developing new varieties with adequate and durable resistance. ARS scientists in Pullman, Washington, identified 19 races, including 2 new races, of the wheat stripe rust pathogen and 10 races, including 1 new race, of the barley stripe rust pathogen from the U.S. 2020 collections. Researchers determined the frequencies and distributions of these races and virulence factors in various epidemic regions. These 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.

Newly identified pathogens threaten chocolate production. Crop losses due to diseases threaten the chocolate industry in the United States. Ghana is the second-largest cacao producer globally, and a disease called thread blight is a new concern for producers in Ghana. ARS researchers in Beltsville, Maryland, working with researchers at the Cocoa Research Institute in Ghana and the University of Ghana, used DNA sequencing to detect four fungi causing thread blight of cacao. A new fungus that had never been found on cacao before was the species most often isolated from infected plants. This information can be used to develop management tools for these thread blight pathogens. Successful disease management will improve conditions in the field, help farmers, and maintain cacao supplies for the chocolate industry.

A new microscopic worm that attacks turf grass in Oregon. Cyst nematodes are microscopic worms that cause billions of dollars of crop losses each year in the United States. Some cyst nematodes infect turf grass and ruin golf courses and other recreational areas. ARS scientists in Beltsville, Maryland, and Oregon State University collaborators used advanced cryo-scanning electron microscopy techniques to visualize cyst nematodes isolated from turf grass in Oregon. Using these techniques, a new cyst nematode species was discovered on turf grass in Oregon and was reported in the journal *Nematology*. This discovery is invaluable for scientists and turf management experts developing new methods to survey turf areas more quickly for this detrimental nematode and implement management practices to reduce turf damage.

Origin of the wheat stem rust race group Ug99. The wheat stem rust pathogen *Puccinia graminis f. sp. tritici* continues to evolve and generate new races that threaten wheat production worldwide. The origin of new race groups of the wheat stem rust pathogen is likely due to recombination events between different races, but little is known about this process. Previous work by University of Minnesota scientists demonstrated that the Ug99 group was formed by the recombination of DNA between two races of *P. graminis f. sp. tritici* that did not involve the normal sexual cycle, which is called parasexual recombination, and identified one of the two races. ARS researchers in St. Paul, Minnesota identified the second race involved in the formation of the Ug99 group. A new method based on single molecule DNA sequencing in individual genes was used to identify one race as the most likely progenitor of the Ug99 group. This new method can be used for identifying parasexual recombination events in cereal rust fungi. Knowledge of how Ug99 originated and has evolved can be used to develop strategies to control and reduce yield losses in wheat due to stem rust.

Biotechnology improves early detection of citrus greening bacteria. Early detection and rapid response are the keys to mitigating citrus greening. Pennsylvania State University and ARS researchers in Fort Pierce, Florida, have used cutting-edge CRISPR/Cas technology to develop a diagnostic test that could enable early diagnosis of citrus greening, or HLB. The new assay can detect the presence of the disease's causal agent—the bacterium *Candidatus Liberibacter asiaticus* (Las)—at a sensitivity level 100 to 1,000 times greater than the commonly used qPCR diagnostic test. The new assay called DETECTR, or DNA endonuclease-targeted CRISPR trans reporter, is compatible with current technology and holds promise for providing a rapid and economical test for citrus greening in the field.

Knowing the genetic material of fungal-like pathogens leads to better identification and diagnostics. *Pythium*, a genus of soilborne plant pathogens, is ubiquitous and is lethal to a wide range of economic crop plants. It also reduces yield and plant vigor. Since different *Pythium* species often have physical features in common, exact identification is difficult by visual inspection. The taxonomy of the genus is unresolved, and more information is needed about relationships between species. An ARS researcher in Salinas, California, collaborated with an Agriculture and Agri-Food Canada scientist to look at the genetic material from every described species and conduct a broad-scale genealogical analysis of the genus to provide a thorough resource to researchers and regulatory personnel. Knowing how the genetic material is put together will lead to simplified species identification and a comprehensive evaluation of taxonomic classifications. It will also elucidate the evolutionary relationship among species, which will support the development of diagnostic markers.

Component 2. Biology, Ecology, and Genetics of Plant Pathogens and Plant-Associated Microbes

The *Septoria tritici* blotch pathogen of wheat senses and responds to light. Scientists know that spores of plant-pathogenic fungi and associated toxin production can be influenced by light. But despite its great economic importance, nothing was known about the photobiology of the *Septoria tritici* blotch pathogen of wheat. ARS scientists in West Lafayette, Indiana, conducted testing on the *Septoria tritici* blotch pathogen to compare the gene expression of cultures grown under white, blue, or red light versus cultures grown in the dark. The results demonstrated that this fungus can sense and respond to different wavelengths of light, and identified some genes that are involved in pathogenicity. These results will be useful to plant pathologists managing *Septoria tritici* blotch on wheat and could help fungicide companies design better control strategies by targeting essential genes for sensing and responding to light. These results were published in *BMC Genomics* less than a year ago but have already been cited by others, which indicates the international interest and potential high impact of this research.

Link found between plant phenols and resistance to grapevine diseases. Fungal diseases and Pierce's disease of grapevines, caused by the bacterium *Xylella fastidiosa*, reduce grape yields. Progression of these diseases depends on specific metabolic plant-microbe interactions involving phenols, which can affect bacterial biofilm formation and fungal lesion lengths, but more information on links between plant metabolism and pathogens is needed. ARS researchers in Parlier, California, discovered that grapevine varieties producing less phenol in response to infection are less affected by fungal canker pathogens. Additionally, scientists found phenolic responses are also linked with grapevine resistance to Pierce's disease. Understanding these interactions will be useful for identifying new grapevine varieties that provide improved resistance to fungal pathogens.

New proteins released from microscopic worms assist in plant infection. Plant-parasitic nematodes are microscopic worms that often inhabit the soil and cause plant diseases. To induce disease, they utilize secreted proteins called effectors. Identifying nematode effectors and understanding how they function in the host root cell is necessary to develop effective and sustainable nematode controls. ARS scientists in Ithaca, New York, discovered for the first time that cyst nematodes have evolved effectors that target the host plant autophagy system. An autophagy system a major intracellular degradation system that has been recently linked to plant defenses against pathogen infection. These studies added new information about the molecular basis of plant-nematode interactions and suggested methods for generating engineered resistance in crop plants, including soybean and potato.

Pathogenicity chromosome identified in widespread strawberry pathogen. *Fusarium oxysporum f. sp. Fragariae*, a host specific fungus that causes Fusarium wilt of strawberry, has become widespread in California, but the genes controlling why this fungus only attacks strawberry plants are unknown. ARS scientists in Salinas, California, compared the genomes and the RNA molecules in a cell of similar fungi, and identified a predicted pathogenicity chromosome involved in causing disease. They determined that this chromosome has moved between similar fungi at least four times and altered their ability to cause disease. This information is a major step toward identifying genes of this fungus that are required to cause disease on strawberry plants and could lead to biotechnology-based strategies for disease control.

Certain nematodes increase severity of cotton wilt caused by a fungus. Cotton wilt caused by the fungal pathogen *Fusarium oxysporum f. sp. vasinfectum* (Fov) is a serious disease throughout the cotton growing regions of the United States. Most races of the *Fusarium* pathogen also require the roots to be infected with root-knot nematodes that form galls on the roots in order to cause the wilt disease.

However, a specific race of the *Fusarium*, known as Fov4, does not require the presence of nematodes to cause a wilting disease on cotton plants. ARS researchers in College Station, Texas, developed a test to look at the interaction between root-knot nematodes and Fov4, and for assessing the subsequent impact of nematodes on disease severity. Their findings using this protocol confirmed that nematodes were not required for Fov4 infection. Results also established that the presence of root-knot nematodes significantly enhanced Fov4 disease severity on many cotton cultivars, including those that were previously shown to be moderately resistant to Fov4. These key results show that if Fov4 spreads to nematode-infested fields, some cotton cultivars that are moderately resistant to Fov4 may not retain that resistance.

Engineering a novel disease resistance trait to a viral soybean pathogen. Genetic-based control using plant disease resistance genes is the most economical and sustainable containment strategy for controlling crop plant pathogens. A team of researchers, including ARS scientists in West Lafayette, Indiana, introduced a novel disease resistance in soybean against a viral pathogen. Results showed that soybeans expressing the modified resistance protein possess robust resistance against multiple pathogens, including a bacterial and a viral pathogen. This work demonstrates the potential to engineer tailored plant resistances to pathogens before effective resistance genes have been identified. This recent work has already been cited by others and generated international interest for this approach to engineering new disease resistance specificities in crop plants.

Sctillometery for assessment of temperature and vapor flux over vineyards. Sctillometery, or laser-based methods for assessing temperature and moisture changes over large heterogeneous vineyards, have been developed by ARS researchers in Corvallis, Oregon. These techniques allow for long term measurement of fine scale changes in vineyards without interfering with vineyard operations. This approach will aid the development of improved predictive models for pest and pathogen spread and disease development. Additionally, this approach will lead to improved assessment of water use and the development of predictive models for plant growth that will aid vineyard managers' decision making.

Bacterial activity improving iron migration toward wheat roots. Iron is essential for plant growth, but it is also toxic to plants at high concentrations. Dryland wheat on the U.S. Pacific Northwest Columbian Plateau enrich bacteria that produce a compound containing phenazine and that can mobilize iron (Fe) and magnesium (Mg). However, more information is needed about how the compounds affect Fe and Mn cycling in the soil around the roots. ARS scientists in Pullman, Washington, in collaboration with a Washington State University student, showed that concentrations of certain forms of Fe were higher in soil with the phenazine-producing bacteria than in soil where the bacteria could not produce phenazine. Although total Fe and Mg uptake into wheat were not different, the phenazine-producing bacteria significantly altered Fe movement toward the roots. These results are important because they show that phenazine producers increased the mobility of Fe derived from soil minerals without producing parallel changes in plant Fe uptake that might cause plant toxicity. This is the first report that directly links significant alterations of Fe-bearing minerals in the soil around the roots to a single bacterial trait and could be important in iron-poor soils.

"Hidden" pathogens can live in plants without causing disease and avoid detection. The movement of plants is the main avenue for the introduction of invasive plant pathogens. Regulatory agencies try to reduce the risk of introductions by conducting import inspections, but limited personnel and the high volume of imported plants make this job difficult. Port inspectors are trained to identify known plant pathogen-host associations, but in some cases plant pathogens may infect plants that are not known hosts and do not cause disease symptoms. ARS scientists in Fort Detrick, Maryland, surveyed

Rhododendron plants in native stands in the eastern United States, cultured microbes from the native plants, and used molecular sequencing methods to identify the microbes. Twenty two percent of the fungal microbes they cultured and identified were reported to be pathogens of other host plants. The researchers then showed that two of the recovered fungi are pathogenic on other host plants. These findings demonstrate that plant pathogens living within non-host plants could escape detection and be transported to new locations where their host is present. This study highlights that plant import inspection methods may not be adequate to detect the importation and spread of new invasive plant pathogenic species.

Validation of two pathogen genes that facilitate disease on barley. Net form net blotch of barley caused by the fungus *Pyrenophora teres f. teres* is a major problem in almost all barley growing regions of the world. It is a particularly important problem in U.S. barley growing regions, regularly causing 5–10 percent yield losses and requiring fungicide applications to control the disease. ARS researchers in Fargo, North Dakota, identified two fungal genes, VR1 and VR2, that contributed to virulence of the disease on the experimental barley line Rika and found mutated versions of these genes reduce disease virulence in Rika barley. VR1 and VR2 are currently being used by barley geneticists to clone the corresponding barley resistance/susceptibility gene(s). This information will be useful to other scientists working to understand necrotrophic, or tissue killing, pathogens of all crop plants, and useful to barley breeders and geneticists breeding resistance to this pathogen.

A fungal protein targets two distinct cell death pathways in wheat. *Septoria nodorum* blotch (SNB) of wheat is caused by the fungal pathogen *Parastagonospora nodorum*. It is a major leaf disease that causes significant yield reductions for wheat growers and is a model system for understanding necrotrophic pathogen-host interactions. Previously, it was believed that there were three *Parastagonospora nodorum* proteins that interacted with the wheat susceptibility genes Snn2, Snn6, and Snn7 to induce cell death. ARS researchers in Fargo, North Dakota, showed that a single protein, SnTox267, targeted all three wheat susceptibility genes to induce cell death, allowing the pathogen to colonize, gain nutrients, and reproduce. Further studies indicated that SnTox267 is exploiting at least two cell death pathways to cause disease. This information is being used by wheat breeders to develop improved varieties that are non-responsive to this fungal protein and thus more resistant to SNB disease.

DNA in the oat crown rust associated with overcoming a crown rust resistance gene in oat. ARS scientists in St. Paul, Minnesota, released high-quality genome sequences of two *Puccinia coronata f. sp. avenae* (Pca) fungi, which causes oat crown rust. These two genomes were used to direct a study of two sets of Pca collections from 1990 and 2015 and analyze changes that may explain the most recent oat crown rust epidemics across the continental United States. This analysis indicated that DNA sequences and traits for overcoming crown rust resistance genes in oat varied significantly between the two collections. Results also identified genomic regions, genes, and DNA markers that might be involved in regional Pca adaptations and the ability of Pca isolates to overcome crown rust resistance genes. This information will assist in development of crown rust resistant oat cultivars.

Genetically distinct groups of *Puccinia triticina* in the United States. Leaf rust caused by *Puccinia triticina* (*P. triticina*) is an important disease of wheat. New races of *P. triticina* that overcome leaf rust resistance genes in wheat cultivars constantly appear in the United States. ARS scientists in St. Paul, Minnesota, tested a total of 159 isolates of *P. triticina* collected from 2011 to 2018 in the United States for traits conferring the ability to overcome leaf rust resistance genes. They tested the isolates on a set of 24 lines of wheat that differ by a single leaf rust resistance gene. The isolates were also genotyped for DNA sequence variation and eight genotype groups were identified. More than 80 percent of the isolates fall into two major groups that were widely distributed across the wheat growing regions of the Great Plains, where hard red winter and spring cultivars are grown, and the eastern and southern regions of the United States, where soft red winter wheat cultivars are grown. Nineteen genomic regions were highly associated with overcoming the resistance of 11 leaf rust resistance genes. This data will provide insight on the evolution and increase of leaf rust races that can overcome the resistance in widely grown wheat cultivars in the United States.

Tracking movement of invasive, disease-vectoring nematode of beech trees. Beech leaf disease is caused by a newly identified nematode pest and can kill both saplings and mature beech trees. In the United States, the nematode has spread from the Great Lakes to the Atlantic Ocean since its emergence. ARS scientists in Beltsville, Maryland, collaborated with scientists from Rhode Island and from Ontario, Canada, and generated DNA marker sequences of the nematode from beech leaf disease samples collected in the United States and Canada. These markers can assist with tracking genetic subpopulations of the nematode and the spread of the disease in North America. These research findings are being used by scientists, growers, regulatory agencies, and extension agencies to effectively monitor and control the spread of this emerging nematode pest.

Pathogen kills raspberries at warmer temperatures than previously expected. A fungal-like pathogen that lives in the soil is known to cause root rot that kills red raspberries in the Pacific Northwest. Due to the nature of this pathogen, it was previously assumed that root rot would be worse in winter, when the soil was cool and wet. Because of this, most disease control measures are implemented from late fall through early spring when these conditions predominate. However, ARS researchers in Corvallis, Oregon, showed that the pathogen is actually more active during the warmer temperatures in late spring and summer when there is less rain. Therefore, disease control might improve if management tactics were implemented during warmer weather, which could dramatically reduce death rates of red raspberries.

Soybean aphids can overwinter on the invasive glossy buckthorn shrub. The soybean aphid is a major pest of soybeans, both in Asia where it is native, and in the United States, where it was introduced. The rapid spread and establishment of the soybean aphid in the United States since its discovery in 2000 was successful because of extensive soybean production in the Midwest and the wide distribution of common buckthorn, its preferred primary winter host. Both common buckthorn and glossy buckthorn are widely distributed in the United States and Canada, but the ability of aphids to overwinter on glossy buckthorn has not been described. ARS scientists in Urbana, Illinois, compared the ability of four soybean aphid biotypes to survive on common and glossy buckthorn. Results showed that nymphs of all four soybean aphid biotypes reached adulthood and produced eggs on glossy buckthorn and that soybean aphid biotypes 2 and 3 males reproduced on both common and glossy buckthorn. This finding suggests that the density of glossy buckthorn in midwestern states may be important in the spread of aphids on soybeans. This work will be of interest to entomologists that study the overwintering of insects, to ecologists, and to soybean producers.

Component 3. Plant Health Management

Managing tomato brown rugose fruit virus. Tomato brown rugose fruit virus (ToBRFV), an emerging and economically important plant virus, has caused serious disease outbreaks on greenhouse tomatoes around the world in recent years. This seed-borne and mechanically transmitted virus poses a serious threat to the $2.5 billion tomato and pepper industries in the United States. Recently, ARS researchers in Charleston, South Carolina, reported an outbreak of ToBRFV on tomato for the first time in the United States. Based on this report, USDA-APHIS issued a federal order to inspect all imported tomatoes and peppers for ToBRFV infection to prevent potential devastation to the U.S. tomato industry. Also, the researchers conducted molecular and biological characterizations of ToBRFV isolates in the United States and developed a highly sensitive real-time PCR detection system for the virus that can also be used for seed health testing. Additionally, several disinfectants that kill the virus and prevent the disease from spreading have been identified and recommended to growers. Furthermore, the researchers screened available tomato germplasm and identified new sources of resistance that are being used in breeding to develop plants that are resistant to the virus. These research findings will provide fundamental knowledge and practical solutions to prevent and protect tomato and pepper crops in the United States and around the world from potential devastation by this emerging and economically important plant pathogen.

New sources of resistance to root-knot nematodes in peanut. The peanut root-knot nematode (PRKN) is a microscopic worm that inhabits the soil. It infects peanut roots and pods and can cause substantial yield losses to peanut growers. Currently, there is only one available source of genetic resistance to this nematode in peanut, and additional sources are needed to improve the durability of resistance. ARS scientists in Tifton, Georgia, and University of Georgia collaborators used a technique known as marker-assisted selection to transfer two new genes that showed resistance to PRKN from a wild peanut species into cultivated peanut. High levels of resistance to PRKN were confirmed in these advanced peanut breeding lines. These new sources of PRKN resistance can be used by peanut breeders to create durable resistance when combined with the previous resistance source.

Screening for sunflower resistance to Sclerotinia basal stalk rot. Using field trials to evaluate sunflower resistance to Sclerotinia basal stalk rot is time consuming and offers limited resolution for identifying resistance. ARS scientists in Fargo, North Dakota, and colleagues at North Dakota State University and Iowa State University developed and validated a new greenhouse method using sclerotinia-infected millet seed to infect single sunflower plants to evaluate basal stalk rot resistance. The new method is time- and space-efficient and allows for disease evaluations in a single year, compared to multi-year, multi-location studies using inoculated field trials. Results from the new method were strongly correlated with field observations. This new method, published in the journal *Plant Disease*, will assist sunflower breeders and pathologists in their evaluation of sunflower genetic populations to improve the rapid identification of genomic regions associated with resistance to basal stalk rot.

Machine learning approaches identify microbial and physicochemical variables related to induction to Prunus replant disease. Prunus replant disease is a serious but poorly understood soilborne disease complex that suppresses tree development, efficient water and nutrient use, and crop yields in tens of thousands replanted almond orchards every year. ARS scientists in Davis, California, used multivariate and machine learning approaches with soil and root data sets to identify strong predictors for Prunus replant disease induction. They found exchangeable potassium and soil pH, as well as abundances of multiple bacterial, fungal, and oomycete taxa, were significant factors. The findings will guide targeted testing to predict Prunus replant disease conditions and introduce management strategies.

Host plant resistance to guava root knot nematode in sweetpotato identified. The guava root-knot nematode (GRKN) is a hyper-virulent nematode and invasive species in the southeastern United States, where it has caused serious damage on a wide range of historically root-knot nematode resistant crops, including sweet potato. ARS researchers in Charleston, South Carolina, screened sweet potatoes from the USDA germplasm collections and identified 20 lines with strong resistance against GRKN isolates from South Carolina and North Carolina. The information on these lines has been published and shared with U.S. sweet potato breeding programs, which are using this information to develop new GRKN resistant sweet potato varieties. These GRKN resistant sweet potato varieties will help stakeholders effectively manage and slow the spread of this emerging nematode pest.

Decision aid for spider mite damage to hop cones. Two-spotted spider mites are common and widespread pests in agriculture and can have a significant impact on hop production. Routine management of spider mites with synthetic pesticides is common in commercial hop production to maintain crop quality and yield. However, the widespread overuse of pesticides is likely because there are no formal guidelines for when the pest is most likely to cause crop damage. ARS researchers in Corvallis, Oregon, and university collaborators identified risk factors for pest outbreaks and developed mathematical risk algorithms to predict when hops are most likely to suffer pest damage. The risk algorithm can form the basis of a decision aid to estimate when and where pest control is warranted, safely reduce unnecessary inputs, and enhance the overall sustainability of the industry.

Combined resistance to soybean cyst nematode and charcoal rot. Charcoal rot and soybean cyst nematode are two infectious root diseases that cause significant yield losses in global soybean production. Identifying lines with combined resistance to both economically important diseases will enable soybean breeders to develop varieties with resistance to both pathogens and help soybean growers obtain more sustainable yields. ARS researchers in Jackson, Tennessee, screened 120 publicly available lines reported to have resistance to one or more races of soybean cyst nematode to determine if they also had resistance to charcoal rot. They identified 12 accessions with resistance to one or two races of soybean cyst nematode that also possessed moderate resistance to charcoal rot. Nine lines had yellow seed coat, which is a desirable agronomic trait. This work has been shared in the journal *Plant Health Progress* and seeds have been made available. The lines are recommended as parents in soybean breeding programs for developing soybean cultivars with combined resistance to charcoal rot and soybean cyst nematode.

New antifungal compounds can reduce postharvest storage fungal growth on peanuts. Agricultural products can be contaminated with fungi at harvest. These fungi can grow during postharvest storage and cause commodity losses. Some contaminating fungi can produce mycotoxins that are harmful to livestock and humans, so it is important to identify safe and effective technologies that reduce fungal contamination of food. An ARS scientist in Peoria, Illinois, and a colleague from Henan University of Technology in China utilized two naturally occurring chemicals produced by plants to completely inhibit growth of fungi present on peanut surfaces. The two chemicals are already utilized in the food industry as flavoring substances, and are generally recognized as safe, but they have not previously been used to control fungi infecting peanuts during postharvest storage. This research is an important demonstration that these safe, volatile chemicals can be used to reduce fungal spoilage of stored peanuts and reduce dependence on synthetic pesticides, which may cause adverse effects on humans.

Green manure soil amendments alter soil microbes and increase vegetable yields. Incorporating crop residue into soil as green manures can improve soil and crop health. Microbes in soil play an important but poorly understood role in this process. An ARS researcher in Salinas, California, demonstrated that different kinds of green manures increase the activity of specific types of soil microbes and can increase or reduce vegetable yields. This research improves our understanding of which soil microbes are responsible for the beneficial impacts of green manures and will help optimize this practice for conventional and organic agriculture.

Peanut seeds produce compounds that inhibit aflaxtoxin formation by fungi. *Aspergillus flavus* is a common fungal species that is widely distributed in nature. It can invade preharvest peanut seeds and produce highly carcinogenic aflatoxins that pose a threat to human and animal health. These aflatoxins are responsible for multimillion dollar losses in all segments of the peanut industry. Under favorable conditions, fungus-challenged peanut seeds produce defensive compounds that can inhibit the invading fungus. ARS scientists in Dawson, Georgia, revealed for the first time that during the peanut-fungus interaction, aflatoxin formation was completely suppressed by these peanut defensive compounds in tested *Aspergillus* strains. This discovery will lead to new genetic approaches based on defensive compound profiles—particularly these aflatoxin-blocking compounds—to control aflatoxin production by fungi.

Powdery mildew resistant watermelon and markers identified. Watermelon is an important vegetable crop grown in 44 U.S. states. Many diseases and pests attack watermelon plants and reduce their yield, which results in enormous monetary loss for growers. One disease, known as powdery mildew, can infest watermelon seedlings and reduce their vigor or kill them. Growers routinely spray pesticides to manage powdery mildew of watermelon, but pesticides pose high risk to human health and environment. ARS researchers in Charleston, South Carolina, developed a watermelon breeding line, USVL531-MDR, as an excellent source of resistance to powdery mildew. Researchers characterized the mechanisms of resistance in USVL531-MDR and then developed a molecular marker to be used in breeding programs to incorporate this resistance into commercial cultivars. These research findings are very useful to seed industry and vegetable breeders for developing new watermelon varieties that have powdery mildew resistance. Research results will also greatly benefit the seed industry, watermelon growers, researchers, and extension agents to effectively manage powdery mildew in watermelon.

New sources of grain mold resistance in sorghum. Grain mold on sorghum causes economic losses totaling hundreds of millions of dollars per year in yield and seed quality. The most effective control strategy for this disease complex is the use of resistant sources. ARS researchers in College Station, Texas, and Mayaguez, Puerto Rico, working with collaborators at Texas A&M University, evaluated the Sorghum Association Panel lines for resistance against three grain mold fungi, individually and in combination. Additionally, scientists used a genome-wide association study to identify annotated gene(s) that may play a role in grain mold resistance. This work identified 18 sorghum lines that exhibited grain mold resistance against the fungi either individually or in combination. Single nucleotide polymorphisms (SNPs) with significant links to grain mold response were also tracked to the specific chromosome location based on the sorghum genome sequence. This accomplishment will provide a platform for testing the effectiveness of each gene listed for sorghum defense against these fungal pathogens.

Development of Phytophthora-resistant rootstocks of walnut and almond. Phytophthora root and crown rot are major threats to almond and walnut production in California. ARS researchers in Davis, California, and University of California, Davis, collaborators used RNA interference (RNAi)-based strategies to accelerate genetic improvement of almond and walnut rootstocks. Three lines were generated that exhibited enhanced disease resistance. A transformation protocol for almonds that was not available prior to this work was developed, and transgenic lines carrying RNAi constructs were generated. The RNAi-based plant disease resistance will minimize and, in some cases, eliminate the need for soil fumigation and fungicide applications. This will dramatically enhance the sustainable production of nut tree crops, reduce environmental impacts of production, and reduce human health risks for the public and agricultural workers.

Development of disease-resistant sugarcane cultivars. ARS scientists in Canal Point, Florida, in collaboration of University of Florida and Florida Sugar Cane League, developed and released disease-resistant, high-yielding cultivars for commercial cultivation by the Florida sugarcane industry. In 2021, the Florida Sugarcane Variety Committee approved the release of five new cultivars. Three are for the Florida muck soils and two are for the Florida sand soils. The disease-resistant cultivars have contributed greatly to the sustainable production of sugarcane in Florida, which produces 20 percent of the sugar consumed in the United States.

New durum wheat lines reveal 25 new potential targets for disease resistance. Fusarium head blight disease of durum wheat is a major constraint to the profitable production of this crop, but genetic sources of resistance to the disease are limited. ARS researchers in St. Paul, Minnesota, identified a total of 25 genes with significantly altered expression patterns when the plant was exposed to Fusarium head blight, indicating they could play a critical role in the mechanisms of disease resistance in wheat. Gene network analysis suggests that single or multiple genes among these 25 are acting to enhance disease resistance in these lines. New sources of resistance would benefit farmers by increasing grain quality and reducing production costs by reducing fungicide applications used to control disease.

Adult plant resistance to wheat stem rust characterized. Stem rust is an important disease of wheat worldwide and new sources of resistance are needed for wheat breeding programs, since new races such as Ug99 have emerged that can overcome stem rust resistance in wheat cultivars. ARS scientists in St. Paul, Minnesota, identified three genomic regions in the wheat line CI 14275 that confer adult plant resistance to stem rust. Wheat lines with the three genomic regions had high levels of resistance that were consistent across environments. Molecular markers linked to the genomic regions can be used to select for wheat lines with adult plant resistance in wheat breeding programs. Adult plant resistance to stem rust in wheat may confer long lasting resistance to stem rust, which is important because emerging races of the stem rust pathogen such as Ug99 can overcome frequently used stem rust resistance genes in wheat.

Developing oat germplasm with long lasting resistance to crown rust. Crown rust is an important disease of oat in the United States. Many new races of crown rust appear every year in the United States. The new races often overcome the resistance in oat cultivars and it is important for oat breeding programs to have sources of long lasting, or durable, crown rust resistance. ARS scientists in St. Paul, Minnesota, conducted genetic studies for several years at various locations with different oat germplasm lines that have durable crown rust resistance. Eleven genomic regions in oat were associated with the durable resistance. DNA markers for each genomic region were also developed. The regions that are associated with durable resistance are being combined in oat germplasm lines using the DNA markers. Oat germplasm with combinations of genomic regions associated with durable resistance can be used by oat breeding projects to develop cultivars with long lasting crown rust resistance.

New gene conferring resistance to stripe rust in wheat germplasm. Stripe rust is best controlled through developing and growing resistant varieties with genes for durable high-temperature and adult-plant (HTAP) resistance and effective all-stage resistance. ARS scientists in Pullman, Washington, completed a study of a gene for durable HTAP resistance originally from wild grass *Aegilops ventricose*. They identified a gene for HTAP resistance and mapped the gene to the translocated part of chromosome from *Aegilops ventricose.* This study shows the usefulness of the wheat varieties carrying the *Aegilops ventricosa* translocation and the importance of combining all-stage resistance with HTAP resistance in developing wheat varieties with durable resistance.