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

# National Program 303 🌱 PLANT DISEASES

**FY 2020 Annual Report**

National Program 303, Plant Diseases, focuses on developing effective disease management strategies that are environmentally friendly, safe for consumers, and compatible with sustainable and profitable crop production. This USDA-Agricultural Research Service (ARS) National Program is conducted in cooperation with related research in other public and private institutions. In particular, NP 303 projects are coordinated with those in ARS' National Program 301 (Plant Genetic Resources, Genomics, and Genetic Improvement) toward the overall goal of crop improvement through increased resistance to biotic and abiotic factors and increased understanding of host-pathogen interactions.

The overall goal of NP 303 is to develop and improve ways to 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 120 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 2020 these funds were distributed across 24 competitive projects to ARS scientists and their collaborators. These projects included diseases that affect avocado, cotton, oat, peanut, pepper, soybean, sweet potato, tomato, and wheat.

The United Nations had declared 2020 as the International Year 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 2020, NP 303 scientists participated in research collaborations with scientists from Argentina, Australia, Austria, Azerbaijan, Belgium, Brazil, Cambodia, Canada, Chile, China, Costa Rica, Czech Republic, Denmark, Ecuador, Egypt, Ethiopia, Finland, France, Georgia, Germany, Ghana, Greece, India, Indonesia, Ireland, Israel, Italy, Jamaica, Japan, Jordan, Kenya, Lithuania, Malawi, Mexico, Morocco, Nepal, Netherlands, New Zealand, Pakistan, Peru, Philippines, Portugal, Russia, Rwanda, Saudi Arabia, Slovenia, South Africa, South Korea, Spain, Sri Lanka, Sweden, Taiwan, Tanzania, Tunisia, Turkey, United Kingdom, and Zambia.

The quality and impact of NP 303 research was evidenced during FY 2020 by the following:
  → 277 refereed journal articles published,
  → 1 new invention disclosure submitted,
  → 11 variety and/or breeding lines released which were evaluated and/or bred for disease/pest resistance, and
  → 1 new patent application filed.

In 2020, an external retrospective review panel was convened to review all facets of NP 303 over the past five years. This panel, consisting of academic and faculty representatives from four different land grant universities, reviewed documented quality and accomplishments from peer-reviewed publications, first disease reports, variety and germplasm releases, national sponsored initiatives, and selected accomplishments including external funding received, mentoring and editorships, international collaborations, and technology transfers such as material transfer agreements and patents. The panel's consensus that, in its composite entirety, research conducted in NP 303 over the past five years is fulfilling the stated goals of improving and expanding knowledge of existing and emerging plant diseases and of developing effective disease management strategies that are safe and economically practical and sustainable.

NP 303 encompasses the following three components in fiscal year 2020:

- *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 2020, this program produced many important discoveries and advances. Select accomplishments are described below, grouped by program component.

## Component 1 – Etiology, Identification, Genomics and Systematics

**Stopping blue mold fungus decay in apples.** Apples are one of the most popular fruits in the United States, and they may be in storage for up to 12 months. During storage, the blue mold fungus, which is difficult to control, may cause the apple to rot, reducing its quality and marketability, so new control methods are needed. ARS researchers in Beltsville, MD, in collaboration with the University of Wisconsin, The Pennsylvania State University, and Dartmouth University, have discovered a way to block the gene in the blue mold fungus that causes apple rot in storage. The apple industry and researchers are using this new knowledge to develop postharvest decay treatments for the blue mold fungus in apples.

**First report of grapevine red blotch virus in Idaho.** Some grapevine viruses are harmful to the U.S. wine grape industry, a $6 billion business. Some of these viruses are detrimental to grapevine health, crop load ratio, fruit characteristics, and ultimately to wine quality, while others cause only minor issues. ARS scientists in Parma, ID, with University of Idaho collaborators, conducted research on grapevine viruses in collaboration with commercial Idaho grape growers. This work is the first report on the presence of grapevine red blotch virus (GRBV) in Idaho commercial vineyards. Multiple years of sampling and testing for GRBV indicate the spread of this virus is limited in Idaho. These findings can be used by the grape industry for vineyard replanting decision making.

**Genome sequencing of beet leafhopper will lead to new gene targets for control.** The beet leafhopper (*Circulifer tenellus*) has been established in the United States for more than a century and transmits beet curly top virus (BCTV). This virus causes economic losses and reduced yields for many western U.S. crops, including tomato, pepper, sugar beet, bean, and other crops. To identify new strategies for control of the beet leafhopper and reduce spread of BCTV, ARS researchers in Salinas, CA, and collaborators at the University of California, Davis, conducted experiments to sequence the genome of the beet leafhopper. This information will facilitate ongoing analysis of gene expression in the beet leafhopper, lead to identification of additional gene targets for potential control of leafhoppers, and provide information for comparative studies with other hemipteran insect pests.

**Public release of the genome sequences of multiple pathogens of cacao**. Cacao diseases reduce cacao production by 25 percent annually. ARS scientists in Beltsville, MD, with collaborators from the Indonesian Cocoa and Coffee Research Institute; the University of Hasanuddin, Indonesia; the University of California, Davis; and MARS, Incorporated, published the genome sequences of four

important cacao pathogens, including the causal agent of vascular streak dieback and the emerging pathogen that causes tip dieback and pod rot globally. In addition, another collaborative effort released greatly improved genome sequences of the causal agent of black pod rot found globally, and the causal agent of black pod rot in Africa. The nature of the pathogen causing vascular streak dieback was unknown and establishing its genetic makeup is critical to understand this disease. This work expands knowledge of the genetic makeup of multiple cacao pathogens, providing new knowledge for scientists developing sustainable and global disease management practices in cacao, and for scientists working with similar pathogens in other crops. These publicly released genomes establish the basis for in-depth analyses of these disease-causing pathogens and will be the basis for global studies of the genetic diversity of these pathogens and their interactions with cacao.

**Creation of genomic database for a major soilborne pathogen.** *Rhizoctonia solani* is a soilborne plant pathogen with a wide host range and worldwide distribution. The exact identification of the different isolates is difficult because no spores are produced. Genomes of 13 isolates from the *Rhizoctonia solani* plant pathogen complex were sequenced, assembled, and annotated, and a genome database was created that is publicly accessible. This database provides information to scientists that is critical for development of identification methods for different isolates within the *R. solani* pathogen complex. Identification and differentiation of the many morphologically similar *R. solani* species within this pathogen complex is necessary because individual species vary in response to plant disease control measures.

**Identification and movement of a nematode causing beech leaf disease in North America.** The American beech tree is a major tree species in North American deciduous forests, and in 2012, the green and yellow stripes of beech leaf disease (BLD) were first discovered near Cleveland, OH. Scientists from ARS in Beltsville, MD, along with USDA Forest Service, the state of Ohio, Holden Arboretum, and the Canadian province of Ontario, identified and described a new subspecies of foliar plant-parasitic nematodes in beech leaves. The scientists demonstrated nematode transmission to beech buds and leaves and described its eastward movement and distribution within North America during different seasons. This potentially lethal BLD nematode is of international concern because it is believed to be an invasive species from Asia, where it causes relatively minor damage. These results are important to pathologists, arborists, and regulators of domestic and international trade who want to contain this nematode and reduce its destruction.

**Phytophthora species identified as contributing to walnut tree death in orchards subjected to river flooding and seepage.** Phytophthora is a soilborne water mold that can infect trees in water-saturated soil and resides in rivers and canals that are used to irrigate orchards. In 2017, catastrophic high-water flows associated with exceptional spring storms occurred in California's Feather and Stanislaus Rivers, inundating adjacent walnut orchards directly and by seepage through levees. Bleeding cankers, which typically appeared to start in the English walnut scions above the graft union, formed in many, but not all, of the flooded orchards. ARS scientists from Davis, CA, sampled the cankers, identified the species of Phytophthora, and confirmed their walnut pathogenicity. The scientists concluded tree death and decline associated with the cankers resulted from infection by Phytophthora. Tree loss in the flooded orchards without cankers may have resulted from root oxygen deprivation and/or as-yet unexplored root disease. Application of systemic fungicides that suppress Phytophthora may be justified in walnut orchards inundated by flooding.

**Invasive boxwood blight fungus came to the United States from two foreign sources.** Since 2011, boxwood blight disease has rapidly spread across the United States, causing widespread damage to millions of dollars of plants in landscapes and nurseries. ARS scientists in Beltsville, MD, developed a new comparative genomics approach to trace the origins of the boxwood blight fungi in the United States. More than 2,000 DNA differences were discovered, showing the pathogen entered the United States twice from different sources. This research is important because it shows how the blight fungus is moving around the world, and is critical for efforts to manage this disease.

**Identifying a citrus virus in apple trees affected by rapid apple decline**. Rapid apple decline (RAD) is an emerging problem of apple production in the northeastern United States. The exact cause of RAD is unknown, although different viruses were found in association with the disease. A virus first described from citrus was identified from RAD-affected apple trees by high-throughput sequencing. A sensitive molecular biology detection protocol for this virus in apples was developed. A total of 55 apple samples were tested, and the virus was detected in 34 of them. The possible role of this virus in causing RAD is currently unclear; however, the finding that apple is a natural host is important. A technology transfer document describing this assay was transferred to the USDA Animal and Plant Health Inspection Service and the Pennsylvania Department of Agriculture for use in quarantine and certification programs.

**Enhanced detection of bacteria associated with citrus greening.** Huanglongbing (HLB) is the most destructive disease of citrus worldwide and in Florida. HLB is caused by a bacterium that cannot be grown in culture. ARS scientists in Beltsville, MD, developed a novel set of assays based on antibodies that recognize the pathogen when it is pressed on a paper-like surface. The sensitive assay produces colored spots and is easily scaled to large numbers of samples. This simple method matches the current urgent need for accurate, sensitive, and high-throughput screening of HLB and may play an important role, especially for plant inspection and quarantine programs.

**Molecular characterization of new viruses infecting camellias.** For decades, virus-like symptoms have been reported from camellias, an ornamental plant that is also grown for tea production and as an ethnobotanical medicine. Four novel viruses were identified by high-throughput sequencing technology in camellias with ringspot symptoms. Using the complete viral genomic sequences, molecular tests were developed to detect these viruses in more camellia samples. Results showed that three of these viruses were frequently detected in camellias. The three common viruses were only seed transmissible, which was revealed by testing seedlings grown from infected plants. These results increased the knowledge of viral camellia diseases. It will help devise disease management and clean stock propagation approaches for camellias, most notably for the ornamental and landscape industry.

**Whole genome sequence of the causal agent of citrus stubborn disease.** A bacterium that is the causal agent of citrus stubborn disease (CSD) has a broad host range. It is endemic in California and causes symptoms often confused with Huanglongbing (HLB). To improve knowledge and diagnostics of the bacterium, seven strains of this pathogen were isolated by researchers in Parlier, CA, from different hosts. Whole genome sequence analysis found the chromosomes to be similar except for inserts of repetitive viral sequences in the central region of the chromosome. Additionally, the bacterium had various numbers of extrachromosomal DNA considered to be plasmids. The

conserved nature of the multicopy viral sequences were used to design new primers for more sensitive detection of the bacterium by real-time polymerase chain reaction assays and are now being used for CSD surveillance.

**Vector and symptom determination of an uncharacterized global maize virus.** In recent years, sequences of many previously unknown viruses were discovered in maize, but biological information about how these viruses are transmitted and whether they caused disease was not known. A new maize-associated virus, often termed maize yellow mosaic virus (MaYMV), was recently discovered and found to be highly prevalent globally (Asia, Africa, South America), and present at very high rates. ARS researchers in Wooster, OH, isolated this virus from other coinfecting viruses in source plants and completed its first biological characterization. They detected it in maize where infection caused leaf reddening symptoms in a diverse panel of maize genotypes. They also found it was transmitted by two aphid species and demonstrated its prevalence in East Africa, particularly in Tanzania and Rwanda. This research characterized potential pathogenicity of a newly identified global virus infecting maize, and the findings on virus isolation and transmission opens research avenues to understanding its impact and epidemiology.

**Genomic resources for berry pathogens.** A specific fungal family harbors a variety of widespread and destructive plant pathogens affecting ornamental plants, forest trees, grapevines, fruit canes and trees. ARS scientists in Beltsville, MD, generated a high-quality genome sequence for a fungal species that infects blackberry and raspberry crops valued at approximately $500 million per year. This research will be useful for mycologists and plant pathologists for developing assays for its targeted detection, developing strategies to control its spread, and to prevent economic losses associated with the disease and those caused by related fungi.

**New bacterial plant pathogen of onions.** Onion production in New York is valued at more than $39 million. Losses due to bacterial diseases can be up to 75 percent in infected fields. There are currently no pesticides that are effective on bacterial rots. ARS scientists in Ithaca, NY, discovered a new species of bacteria responsible for an onion disease in New York state. Using genomic information, they determined this species of bacteria was not previously known to exist in the United States. This information is useful for USDA's Animal and Plant Health Inspection Service for monitoring the introduction and spread of plant disease-causing bacteria in the United States.

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

**DNA sequence resources for bacterial plant pathogens of economic importance.** The pathogen that causes almond leaf scorch and olive quick decline and the pathogen that causes citrus greening have significant impacts on crop production worldwide. DNA sequences from these pathogens can be used for detection, surveillance, and disease tracking. ARS scientists in Parlier, CA; Byron, GA; and collaborators in Brazil used next-generation sequencing technology to create DNA sequence resources for pathogen strains associated with olive in California and with pecan in Georgia, and to fully sequence for the first time a citrus greening pathogen strain from Brazil. This new DNA sequence information is valuable for development of new disease management and diagnostic strategies and for pathogen detection and quarantine applications.

**Spread of citrus tristeza virus in central California.** Quick decline (QD) of citrus on sour orange (SO) rootstock is caused by citrus tristeza virus (CTV) and has killed millions of citrus trees on SO rootstock worldwide. The California Department of Food and Agriculture (CDFA), through grower pest control districts, developed quarantines that evolved into two disparate zones in central California. In Zone 1, abatement, including aphid vector control, annual CTV surveys, and selective eradication of potentially severe strains of CTV, is mandatory; in Zone 2, no abatement is practiced. ARS researchers in Parlier, CA, compared CTV spread and symptoms of disease in both quarantine zones and found annual CTV spread was 0.39 and 1.5 percent, respectively, in Zone 1 and Zone 2. Spread of mild strains was 11 times greater than severe strains, suggesting that aphid control and abatement of severe strains was limiting spread of severe strains of CTV. This data is being used by CDFA, pest control districts and the University of California Lindcove Research and Extension Center on decisions to manage CTV.

**Epidemiological models predict the spread of two severe citrus diseases by hurricanes.** Asiatic citrus canker (ACC) and citrus black spot (CBS) are two serious diseases that cause economic damage and are severe impediments to international trade of citrus as a commodity. ACC, caused by a bacterium, and CBS, caused by a fungus, are both dispersed by rain splash. In the presence of hurricanes, such rain splash can be spread over many miles. In 2017, Hurricane Harvey in southeast Texas and Hurricane Irma in southwest Florida potentially spread ACC and CBS, respectively. ARS researchers in Fort Pierce, FL, adapted and extended a previously developed hurricane dispersal model to address both diseases and predict where these infections might have spread due to the hurricanes. The results of these model predictions were presented to the appropriate regulatory agencies and science advisory committees. ARS researchers also developed risk-based surveys to target areas rejected by the hurricane models. As a result, Texas and Florida state regulatory agencies in collaboration with USDA's Animal and Plant Health Inspection Service have deployed the surveys for early detection of potential spread of these diseases in both states.

**Large suction traps lead to better understanding of soybean aphid pests.** The abundance of soybean aphids and other insect species that move through the air and make up the aerobiota in the central United States vary temporally and geographically. The ability to predict damage caused by agricultural insect pests is dependent on detailed information on their abundance and impacts on crops linked to contemporaneous climatological and meteorological factors. ARS scientists in Urbana, IL, collaborated with state researchers, extension specialists, and agriculturalists to analyze insects captured in suction traps located in nine Midwest states. The suction trap data and its samples have generated publications on modeling distribution and migration patterns for soybean aphids, providing new reports of aphid species beyond their known distributions. These data also increased information about the distribution of other insect species, such as mosquitoes and thrips. The collected insects were stored, used for past research, and will serve as a foundation for future research. These data are of interest to researchers working to reduce crop damage from insect pests, and economists and population biologists who are interested in modelling agricultural production systems.

**New detection methods identify and quantify viruses affecting U.S. cucurbit crops.** In recent years, numerous whitefly-transmitted viruses have emerged in the major U.S. cucurbit production regions, leading to reduced yields, and in some areas, significant reductions in crop production.

Many of these viruses produce similar symptoms in infected plants, making resistance evaluations difficult. ARS scientists in Salinas, CA, developed molecular methods to efficiently identify four different viruses that commonly co-infect U.S. cucurbit crops and developed a separate system to quantify different amounts of the four viruses. These methods are being used by ARS in Salinas and other laboratories throughout the United States to determine virus distribution and regional alternate host plants, as well as for evaluating virus resistance in plants.

**Pathogen species shift helps explain control failures for root rot of rhododendron.** Despite 90 years of research, Phytophthora root rot remains a serious problem for the $42 million rhododendron industry. There are no recent damage estimates to determine the extent of the problem, disease control tactics often fail, and little is known about which pathogen species are currently causing the disease. ARS researchers in Corvallis, OR, determined that root rot causes an estimated $6 million in losses (15 percent of crop value) and identified weak points in the production cycle where Phytophthora pathogens become established. In addition, a new species is now more common than the species previously thought to be the main pathogen based on research from 40 years ago. These results may explain why disease control often fails, as the tactics designed to control rhododendron root rot may not be effective for the more prevalent new species.

**Development of a new plant virus-based vector for potential pharmaceutical production.** Pepino mosaic virus (PepMV) is a plant pathogenic virus on tomato that can cause mild or severe symptoms depending upon the strain. Plant virus-derived vectors are excellent tools for expressing and producing recombinant proteins in plants that have the potential to be used in developing pharmaceutical products. The currently used vectors have low stability in plants, variable expression in plant parts, and slow production of protein. ARS research conducted in Beltsville, MD, developed a PepMV-based vector from a mild strain that was a suitable expression tool with enhanced performance and stability and less severe symptoms in tobacco plants compared to the current vector. The PepMV vector also had earlier and more rapid expression, and higher concentrations of foreign recombinant proteins. The developed vector is a promising choice for biotechnology as a rapid turnaround for producing plant pharmaceutical products and other proteins in plants.

**Blueberry as a potential host for bacterium that causes almond leaf scorch and Pierce's disease of grapevine.** The bacterium that causes disease on numerous crops, with strains that infect grapevine and almond in California, had not been assessed for its risk to blueberry in California, although strains found in the southeastern United States cause significant disease in highbush blueberry. ARS scientists in Parlier, CA, evaluated susceptibility of southern highbush blueberry cultivars to California bacterium strains, as well as the ability of glassy-winged sharpshooter to transmit the pathogen between blueberry plants and grapevine. This study found the blueberry cultivar 'Emerald' is more susceptible to California strains than four other cultivars tested, and the study found that glassy-winged sharpshooter can acquire the bacterium from infected blueberry plants. This information is essential for understanding the epidemiology of diseases caused by this bacterium in California, and in evaluating disease risk for expansion of highbush blueberry acreage in areas where this pathogen is problematic.

**Characterization of genes that affect transmission by the glassy-winged sharpshooter of the bacterium that causes Pierce's disease of grapevine.** The bacterial causal agent of Pierce's disease of grapevine is transmitted between grapevines by the glassy-winged sharpshooter. While extensive information is available on the pathogen-grapevine interactions at the biological and molecular levels, little is known about the pathogen-vector interactions, especially as they pertain to transmission efficiency of the pathogen. ARS researchers in Parlier, CA, created several virulence gene mutants of the pathogen and found that mutations altered transmission processes, increasing initial adhesion, multiplication, and long-term retention of the bacterium in insect mouthparts. This study provided new information important for understanding pathogen-vector interactions during the transmission process, which will facilitate identification of key target genes that may serve as a gene-based control method to mitigate pathogen spread in vineyards.

**A fungal pathogen targets a wheat gene, resulting in improved colonization of the leaf.** Septoria nodorum blotch (SNB) of wheat is a major leaf disease that causes significant yield reductions for wheat growers. The pathogen uses necrotrophic effectors (NEs) to induce disease on wheat. ARS scientists in Fargo, ND, previously identified a new NE and its genetic target in susceptible wheat. Scientists labeled various strains of the pathogen with red fluorescent protein and used laser confocal microscopy to show the pathogen secretes the newly identified NE, which targets the susceptible wheat gene, allowing the pathogen to fully colonize the wheat leaf. This information is critical to wheat breeders and geneticists trying to breed resistance to this pathogen, as well as scientists working on other agriculturally important necrotrophic pathogens. This NE is currently being used by geneticists and breeders to eliminate this susceptibility gene.

**Unique new stem rust populations.** ARS researchers in St. Paul, MN, identified a unique and highly diverse stem rust population in Spain through collaboration with the Global Rust Initiative. Analyses of many isolates revealed this population has numerous novel virulence combinations that are highly unique and potent. For instance, most of the isolates possess greater virulence profiles than Ug99 races, attacking many resistance genes currently used in breeding for Ug99 resistance. These isolates also process virulence to two other genes, a rare and highly significant virulence combination that was previously known to occur only in the Ug99 race group. Detection of such potent rust populations indicate the continual need for vigilance as well the need for the identification and deployment of new plant resistance genes to combat this pathogen.

**Basic research tools developed to understand maize viruses.** Infectious clones are a foundational research tool for understanding virus biology, including maize virus gene function and infection interactions that lead to disease. The ability to genetically manipulate and replicate viruses in the laboratory is essential for basic virology research and can also be used to understand maize host plant gene functions. However, these tools are lacking for many maize viruses. ARS scientists in Wooster, OH, developed new and improved methods for conducting virology research and studying virus-based functional genomics with maize viruses. They improved the infectivity and sequence stability of a laboratory clone of an important virus that infects U.S. maize, developed infectious clones and an effective delivery methodology for difficult-to-deliver maize viruses, and developed clones that efficiently silence host maize genes, enabling the study of their functions. Infectious clones of these viruses and derivative tools have been requested by multiple national and international laboratories for research purposes, and a subset are currently shared with national laboratories to better understand plant or virus biology and disease.

**Fiber and grain crops are hosts of root-knot and cyst nematodes.** Plant-parasitic nematodes cause an estimated $10 billion dollars of crop losses each year in the United States and $100 billion dollars globally, but little is known of their host range. ARS scientists in Beltsville, MD, in collaboration with Egyptian scientists, discovered that root-knot and cyst nematodes could infect several cotton, sesame, oat, and wheat cultivars as well as weedy grasses. These significant findings provide the first evidence that important fiber and grain crops are susceptible to nematodes, and will be used by researchers, growers and extension personnel involved in root-knot and cyst nematode research and control.

**Identifying weed hosts leads to better virus management strategies for lettuce.** Lettuce production in the Salinas Valley of California accounts for more than 50 percent of annual U.S. lettuce production. However, over the past several years, there have been increasing reports of impatiens necrotic spot virus, a thrips-transmitted virus that severely impacts lettuce production. In 2019, several growers reported up to 100 percent crop losses due to this virus. Few insecticides can manage thrips and there are no existing methods for managing the virus. ARS researchers in Salinas, CA, identified important weeds that can serve as virus reservoirs during the winter months, which is during the off-season when lettuce is not grown. This has resulted in improved weed management by growers and by the California Department of Transportation, which is managing weeds along major highways throughout the Salinas Valley.

**Using wild potato species for resistance breeding to potato cyst nematodes.** Potato cyst nematodes, including the golden nematode and the pale cyst nematode, are quarantine pests important to agriculture. Deploying resistant plant cultivars is the most effective and sustainable means for combating potato cyst nematodes. ARS scientists in Ithaca, NY, identified a list of potato clones showing broad and robust resistance against multiple kinds of potato cyst nematodes. Potato breeders are using these wild clones to generate new nematode-resistant potato cultivars in the United States.

**Manipulating cotton to enhance resistance to Fusarium wilt.** Terpenoid compounds derived from two biochemical pathways in cotton play an important role in defending cotton plants against herbaceous insects, animals, and pathogens. Both pathways share a common initial biosynthetic step that can be manipulated to affect each pathway. Using a molecular technique known as RNAi suppression, ARS researchers at College Station, TX, blocked one of these pathways and increased resistance to Fusarium wilt pathogens. Comparison of terpenoid contents in the roots of RNAi suppressed and non-suppressed plants revealed increased production of RNAi plants' antifungal compounds. The concentration of these antifungal compounds found in the roots of RNAi suppressed plants were shown to inhibit the growth of Fusarium by more than 93 percent. The increased induction of this pathway likely enhanced resistance to Fusarium in the RNAi suppressed plants. Genetic manipulation of these two major cotton chemical defense pathways may provide a new approach to increase host resistance to Fusarium wilt pathogens in cotton.

**Genes associated to avirulence in the wheat stripe rust pathogen identified.** Understanding the molecular mechanisms of virulence is important for the control of stripe rust. ARS scientists in Pullman, WA, published the first two papers that identified 16 avirulence genes in the wheat stripe rust pathogen. Avirulence is a pathogen's inability to actually produce any pathological effects. Of

these, 4 loci clustered in a small genome region (less than 200 kb). Additionally, 62 candidate genes were identified that are highly associated with these 16 avirulence loci. These studies provide the genomic bases foundation for understanding the molecular mechanisms of the pathogen's evolution and resources for cloning avirulence genes for further study of the plant-pathogen interaction.

**Risk model and resulting risk-based survey direct California program to combat Huanglongbing.** Huanglongbing (HLB) has devastated the Florida citrus industry and is now threatening the industry in California. Although HLB has not been detected in commercial groves, infected trees were found in four counties in California in residential areas. California currently has 75–100 surveyors dedicated to state-wide surveys, but they cannot reach all commercial and residential properties. ARS researchers in Fort Pierce, FL, developed a risk-model that uses 10 risk criteria to rank each 1 square mile area in California that deployed a risk-based survey to target and optimize survey efforts and capture the seasonality of disease development. Now in the 9th year of deployment in California, the risk-based residential and commercial survey methods for Asian citrus psyllid (ACP) and HLB have been shown to be highly successful and have become the mainstay of the large-scale statewide survey. The survey continually detects new infections in southern California that exceeded 2,100 at the end of March 2020. Infected trees are immediately removed and a quarantine is placed surrounding the detection source with delimiting response protocols. At present, the survey is done visually with PCR confirmation of suspicious trees; however, canine detection will likely be integrated into the risk-based survey.

**Genes identified in resistant durum wheat are involved in plant defense.** The plant disease Fusarium head blight (FHB) is an important constraint to profitable production of durum wheat in the upper 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, MN, studied differences in the genes expressed by disease susceptible lines and a newly developed M4 line that has moderately high FHB resistance. The resistant M4 line activated many categories of genes associated with an active plant disease defense response during infection. This new native source of FHB resistance in durum wheat will be beneficial in breeding resistant cultivars.

**Identifying the mechanisms of preharvest aflatoxin resistance in peanut.** Natural genetic resistance to aflatoxin accumulation in peanut seeds is quantitative. Aflatoxins are produced as a secondary metabolite by the Aspergillus fungus. Current breeding methods for developing aflatoxin-resistant peanut cultivars are genetically inefficient due to limited understanding of the molecular mechanisms underlying aflatoxin formation in peanut seeds. ARS researchers in Dawson, GA, identified and validated aflatoxin-resistant peanut genotypes using a novel non-destructive method of screening they developed. This germplasm was further used to characterize gene expression changes in the peanut-Aspergillus interaction of susceptible vs. resistant genotypes using RNA sequencing. Genes responsible for producing antifungal compounds were among the plant genes that differentially responded to Aspergillus infection. These findings are expected to have immediate application in pre-breeding/breeding and assessment of resistant candidates at pre- and post-harvest.

**Large number of genes resistant to stripe rust in U.S. wheat varieties identified and mapped.**
Stripe rust is best controlled through growing resistant varieties, but stripe rust resistance genes in most U.S. wheat varieties are not evident. ARS scientists in Pullman, WA, published two papers on identification and mapping of 37 genes in 616 spring wheat entries, and 51 genes in 857 winter wheat entries, using a genome-wide association analysis approach and molecular markers for previously reported genes. The varieties included commercial varieties, breeding lines, and genetic stocks developed or used in the United States for resistance to stripe rust. They determined the frequencies of these genes in varieties from different regions and determined how gene numbers affect resistance. Their results identify the effectiveness of individual genes and the importance of pyramiding different resistance genes. The resistant varieties, genes, and their markers identified in these studies are valuable for growers to enable selection of varieties with adequate levels of resistance to stripe rust. The new genetic information is also used by breeders to select resistant varieties to cross and markers to use for developing new wheat varieties.

**Maize chlorotic mottle virus resistance introgression into useful genetic backgrounds for agriculture.** Maize lethal necrosis (MLN) is a globally emerging virus complex severely impacting yield and food security. It is caused by co-infection of any endemic maize virus with the emerging maize chlorotic mottle virus (MCMV). Resistance was previously identified for maize viruses, but ARS researchers in Wooster, OH, recently identified MCMV tolerance traits in corn. To further enable usefulness of this identified resistance, advancing its introgression from tropical source germplasm to usable breeding germplasm is ongoing. The resistance traits will be fixed in desirable germplasm and evaluated for MCMV tolerance prior to release. This germplasm is important because maize in East Africa is predominantly white endosperm and used for human consumption, and MCMV resistance source from white endosperm will speed the breeding process.

## Component 3 – Plant Health Management

**Importance of antifungal compounds secreted from a bacterium used as crop protection agents.**
Some *Bacillus* species are the most successful bacteria developed as biocontrol agents for control of plant pathogens, and also serve as important human, livestock, and aquaculture probiotics. ARS scientists in Peoria, IL, discovered that distribution of antifungal compounds produced by these bacteria can predict their taxonomy. They also discovered this class of antifungal compounds evolved from one compound into six related antifungal compounds. Understanding how these antifungal molecules originated and evolved provides new insights into the interactions between crop protection bacteria and the plant pathogenic fungi they control and should enhance the use of these beneficial bacteria as crop protection products.

**Biological control of coffee berry borer in Puerto Rico.** Coffee production in Puerto Rico, with an estimated annual value of $100 million, is under significant threat from the invasive coffee berry borer. ARS scientists in Beltsville, MD, in collaboration with university researchers in Puerto Rico, conducted field experiments to see if different fungal strains that are pathogenic to insects reduced populations of coffee berry borer. They compared the effects of a commercial fungal strain (Mycotrol) and local strains of an insect pathogenic fungal species. Two of the local strains, applied individually and as a mixture, were either equally or more effective than Mycotrol in suppressing coffee berry borer infestations and generally persisted in the environment longer than the

commercial strain. Determining the potential of local strains of fungal species for managing populations of this invasive and economically destructive insect will contribute to improving coffee yield and quality in Puerto Rico.

**Identification of antifungal compounds from bacteria to control onion rot.** Soft rot, slippery skin, and sour skin are diseases of onions caused by bacteria. There are currently no available pesticides for bacterial rots. Many bacteria naturally produce biochemicals that inhibit the growth of fungal plant pathogens, and ARS scientists in Peoria, IL, identified a soil bacterium from the ARS Culture Collection with this ability. In liquid culture, the bacterium secreted biochemicals that inhibited growth of many fungal plant pathogens and that were effective against a fungal pathogen of onion grown in greenhouse assays. These biochemicals were identified as edeines, a class of antibiotic metabolites that exhibit broad antibacterial, antifungal, and bioherbicidial activity. This work increases our knowledge of this soil microbe's important biological activity. In addition, comparative genomics research determined that gaining the ability to produce edeines played an important role in the evolution of the bacteria in this genus (*Brevibacillus*). Therefore, these compounds may be more useful in postharvest or other unique applications than traditional crop protection treatments.

**Combining herbicides with biofumigants for improved pest and disease control.** The loss of ozone-depleting methyl bromide has left growers with few soil fumigants options, particularly for buffer zone areas where conventional fumigants cannot be applied due to potential for bystander exposure. Alternatives to methyl bromide are urgently needed for both conventional and organic crop production. ARS researchers in Fort Pierce, FL, conducted field trials using a combination of herbicides and a newly registered pre-plant biofumigant with the active ingredient allyl isothiocyanate for tomato and bell pepper production in Florida. The treatments resulted in broad-spectrum control of pathogens and weeds that equaled control achieved with methyl bromide, including reducing the incidence of bacterial wilt of tomato, root-knot nematodes, and nutsedge weeds. These combined herbicide and biofumigant treatments provide a greatly needed pest and disease control option for both conventional and organic crop production in Florida and elsewhere.

**Precision thermotherapy lowers leaf spot on strawberry.** A bacterium that causes angular leaf spot (ALS) of strawberry can be found routinely on strawberry nursery stock. Heat treatment has been shown previously to be effective for managing ALS on nursery stock in small-scale experimental trials. ARS researchers in Fort Pierce, FL, designed, built, and tested a commercial-scale precision thermotherapy unit (PTU) for applying a new thermotherapeutic protocol on strawberry nursery stock that combined a conditioning thermal treatment with an eradicative thermal treatment. Several trials were conducted in cooperation with commercial nurseries to determine the impact of thermotherapy on plant health and on the natural development of ALS. Overall, precision thermotherapy had a negligible effect on plant growth and yield. ALS, when it occurred, was always lower in thermotherapy-treated plots. Successfully scaling up thermal treatment of nursery stock to commercial levels could have a unique impact on controlling pests and diseases while simultaneously reducing or eliminating pesticide use against a broad range of threats to sustainable strawberry production.

**Integration of linkage blocks of wheat stem rust (Ug99) resistance genes.** Linked disease resistance genes are a great tool for gene stewardship that serve to extend the life of resistance in modern

varieties. ARS researchers in St. Paul, MN, have combined multiple linked wheat stem rust resistance genes effective against Ug99 and backcrossed these linkage blocks into conventional wheat germplasm. Two resistance genes were combined on one chromosome arm and two other genes were combined on a different chromosome arm. The two linkage blocks were backcrossed into hard red spring wheat cultivars previously released by the University of Minnesota and South Dakota State University. The Ug99-resistance-enhanced lines have been used in the crossing blocks of these two breeding programs for the development of wheat cultivars for the United States with multiple Ug99-resistant genes.

**Phenological-timed fungicide applications have improved disease management of grape powdery mildew and reduced costs.** Grape powdery mildew is difficult and expensive to control in the western United States and accounts for 78 percent of the pesticide use in grapes. In addition, fungicide resistance has been an increasing problem, making management more difficult. ARS researchers in Corvallis, OR, demonstrated that numerous fungicides were mobile—moving through the plant—through at least one of three different mechanisms tested. These data were then used to design disease management programs that targeted mobile fungicides to critical crop growth stages. Applying micronized sulfur every 14 days all season, and timing mobile fungicides to late bloom through BB-sized berries resulted in excellent disease management. This method reduces fungicide costs to more than $90/acre and uses only two non-sulfur applications per season. These data will aid in developing improved fungicide resistance and mitigation strategies.

**Identification of cultural and biological control methods for insect vectors of grapevine red blotch virus.** The threecornered alfalfa hopper was recently shown to be a vector of grapevine red blotch virus, an economically important pathogen of wine grapes. There are no pest management recommendations targeting the vector. The risk of threecornered alfalfa hoppers establishment in vineyards may be reduced by planting cover crops that are not hosts for the pest, and by releasing commercially available biological control agents that consume the hoppers. ARS researchers in Parlier, CA, determined that orchard grass, creeping red fescue, fawn tall fescue, hard fescue, and California poppy did not support adult threecornered alfalfa hopper survival or reproduction, and determined that green lacewing larvae readily consumed all juvenile life stages of the hopper. Results can be used by grape growers when selecting cover crop species and biological control agents that may aid in reducing threecornered alfalfa hopper populations in vineyards.

**Evaluating soybean lines for incidence and severity of foliar diseases in Zambia and Malawi.** Soybean production in sub-Saharan Africa is important to small-plot farmers. However, few high-yielding disease-resistant soybean cultivars are available that are adapted to the region. ARS researchers in Urbana, IL, collaborated with other national and international agencies and research groups to improve overall performance of soybean breeding stocks in Africa. As part of the Pan African Soybean Variety Trials, high-yielding cultivars from Brazil and the United States were evaluated in Zambia and Malawi for three bacterial diseases, six fungal diseases, one oomycete, and viruses. While the cultivars were significantly vulnerable to infection from most of the diseases tested, they were not as susceptible to infection from soybean rust and target spot. This work provided a benchmark for the status of soybean diseases in sub-Saharan Africa and will be important to soybean researchers worldwide, as well as breeders, pathologists, and farmers in Africa.

**Development of Phytophthora-resistant rootstocks of walnut and almond via RNAi-based method.** Phytophthora root and crown rot is a major threat to almond and walnut production in California. In collaboration with University of California, Davis, ARS researchers in Davis, CA, employed RNA interference (RNAi)-based strategies to accelerate genetic improvement of almond and walnut rootstocks. RNAi transformation vectors containing selected Phytophthora gene segments were constructed and delivered to plants, and 100 transformants were created. ARS researchers developed a disease resistance bioassay to identify three walnut transformant lines showing improved Phytophthora resistance. Almond transformants were also generated and are being examined in disease resistance bioassays. 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 while reducing its environmental impact and human health risks for the public and agricultural workers.

**Characterization of soil microbial communities responsive to anaerobic soil disinfestation (ASD), an alternative to chemical-based soil fumigation.** ARS researchers in Davis, CA, assessed soil microbiome shifts in response to different ASD carbon source amendments. Results from field and greenhouse trials showed rice bran and tomato pomace elicit highly similar microbial communities and exhibit similar efficacy at controlling targeted plant pathogens. Analyses of these communities point to the potential importance of nitrogen fixation and denitrification in altering nutrient dynamics in ASD-treated soils. Tomato pomace is a more cost-effective carbon source for ASD than rice bran, and may facilitate ASD adoption as an alternative to pre-plant chemical fumigation of soil to control plant pathogens.

**Updating the impact of mycotoxins on sesame yields in the Mississippi Delta.** Sesame is increasing popular with U.S. consumers, so more acreage in the Mississippi Delta has been planted with sesame. ARS scientists in Stoneville, MS, conducted sesame field trials in the Mississippi Delta and found that production practices and crop variety did not notably affect yield or sesame seed contamination with mycotoxins or toxigenic fungi. They also collected samples of the primary fungal species that cause aflatoxin and noted that nearly all lacked the biosynthetic gene clusters that produce toxins. The reason appears to be prior applications of Afla-Guard®, a non-toxigenic strain of the fungus used for biological control, in fields near the experiment and collection sites. The results of this study indicate pathogenic fungi and mycotoxins may not be a concern for safe sesame production and consumption. This information will be useful to public and private sector entities, including ARS, academia, industrial grain producers, and growers currently producing the sesame crop.