National Program 303 • PLANT DISEASES
FY 2013 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 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. Fiscal year 2013 saw the merger of National Program 308 (Alternatives to Methyl Bromide for Pre-Plant) into other programs – the new NP 303 Component 4 encompasses research previously in NP 308.

The overall goal of NP 303 is to develop and improve ways to reduce crop losses caused by plant diseases, while safeguarding the environment. To this end, projects in this national program aim to reduce the impact of diseases on yields, product quality or shelf-life, aesthetic or nutritional value, and potential contamination of food and feed with toxins.

Management of plant diseases is essential for providing an adequate 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 with minimal increases in 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 70 projects located in 19 different states. Most of the more than 120 scientists working within this national program are specialists in plant pathology and/or nematology. Significant contributions to NP 303 also come through multidisciplinary teams that include geneticists, agronomists, botanists, horticulturists, physiologists, soil scientists, entomologists, chemists, and microbiologists.

NP 303 is comprised of the following four components:

- *Diagnostics, Etiology, and Systematics*
- *Biology and Epidemiology of Plant Disease*
- *Plant Health Management*
- *Alternatives to Preplant Methyl Bromide Soil Fumigation*

Together, these components include research to understand and control plant diseases and to develop strategies for disease management and control that enhance agricultural production and value. During fiscal year 2013, this program produced many important discoveries and advances. Some of these are described below, grouped by program component.

Component 1 – Diagnostics, Etiology, and Systematics.

Molecular diagnostic assay for wheat stem rust Ug99 strains. Strains of the wheat stem rust pathogen in the group Ug99 are threats to wheat production worldwide, and while these strains are not yet in the United States, U.S. wheat varieties are vulnerable to Ug99. Until now, the only way to distinguish Ug99 strains from other forms of wheat stem rust was to put the fungus spores on wheat plants and wait for disease to develop. ARS scientists in St. Paul, Minnesota, have developed a two-stage assay based on fungal DNA to distinguish among rust strains. The first stage determines if the sample belongs to the Ug99 strain group, while the second stage predicts the specific strain. This assay is currently being used to track the movement of the Ug99 in Africa, where the disease is endemic. Deployment of this assay in the United States would greatly enhance growers' ability to detect and identify any Ug99 introductions and to provide information for responding to potential outbreaks.

Flat mite identification tool on the Web. Flat mites, such as false spider mites, red palm mites, citrus mites, and peacock mites, are devastating pests on citrus, tea, bananas, coconuts, date palms, olive, eucalyptus, and ornamental palms. In addition to directly causing damage, these mites also vector plant diseases, including citrus leprosis virus. Accurate identification of these mites is the first step in controlling them. ARS researchers in Beltsville, Maryland, in collaboration with the USDA Animal Plant Health Inspection Service (APHIS) developed an interactive on-line identification key with descriptors and numerous images using light microscopy and low temperature scanning electron microscopy. Since its launch 1 year ago, more than 123,800 visitors from 180 countries have accessed the Web site. This tool has enabled correct identification by farmers, extension agents, state and university researchers, government agencies, and APHIS quarantine specialists controlling mites and plant disease vectored by mites.

Method for the detection of Triticum mosaic virus developed. The availability of diagnostic methods for rapid, sensitive, and large-scale detection of viruses is crucial for the management of plant viral diseases. Diagnostic methods for Triticum mosaic virus, a recently reported virus from the Great Plains region, are not available. ARS scientists in Lincoln, Nebraska, produced polyclonal antibodies against the bacterially expressed coat protein of Triticum mosaic virus. In enzyme-linked immunosorbent assays (ELISA), these antibodies detected various isolates of Triticum mosaic virus in crude plant sap, but not in extracts of healthy or Wheat streak mosaic virus-infected plants. The availability of Triticum mosaic virus antibodies would provide a high-throughput ELISA-based detection method and germplasm screening in wheat breeding programs. A biotechnology company is in the process of commercializing these antibodies for the development of a Triticum mosaic virus diagnostic kit.

New species of cyst nematode. Cyst nematodes are an important group of plant pathogens because they damage the roots of many kinds of crop plants worldwide and can halt trade between countries because they may be regulated by quarantine. ARS scientists in Beltsville, Maryland, and in Corvallis, Oregon, in collaboration with Oregon State University, described the detailed anatomical and molecular features of a new cyst nematode, *Globodera ellingtonae*, discovered in soil samples collected during surveys to detect potato cyst nematodes. The new nematode, which was first found in Powell Butte, Oregon, shares key anatomical features with two potato cyst nematodes species of regulatory concern, the pale potato cyst nematode and the golden nematode. The newly described species can confound accurate diagnosis of potato cyst nematodes because current molecular tests are not set up to identify it. The morphological and molecular data describing this lookalike species will help scientists, regulators, and extension agencies to more accurately identify and prevent the spread of potato cyst nematodes.

Improved diagnostic method to identify tuber necrotic strains of Potato virus Y (PVY). Tuber necrotic strains of PVY are emerging in the United States and have the potential to become a major quality disease issue for the U.S. potato industry, threatening farm income and export options. In collaboration with university scientists, ARS scientists in Ithaca, New York, have developed new knowledge of the specificities and shortcomings of commercially available diagnostic reagents for PVY. This led to improved testing protocols that eliminate false positives and allow detection of all variants within the tuber necrotic strain of PVY. Protocols were transferred to state and federal partners that conduct product testing and regulate interstate and international commerce of potatoes.

Causative agent of Red Blotch disease of grapevine. Red Blotch disease causes significant vineyard losses due to reduced yields and grape quality. ARS scientists in Davis, California, discovered and characterized a new Gemini-like virus, associated with this disease, named Grapevine red blotch-associated virus (GRBaV). Epidemiology of Red Blotch disease suggests GRBaV exhibits insect-mediated transmission. The scientists developed Red Blotch-specific DNA primers to detect and quantify the virus. These DNA primers are now widely used by both diagnostic testing services and grapevine virologists around the world including in the United States, Australia, Canada, France, Italy, New Zealand, and South Africa.

Component 2 – Biology and Epidemiology of Plant Disease.

Natural plant molecules disrupt nematode development. Safe strategies for managing plant-parasitic nematodes should effectively control these target pests while having minimal impact upon the environment and non-target species. Using the most economically important plant nematodes in the United States, the soybean cyst nematode and the root-knot nematode, ARS scientists in Beltsville, Maryland, found that plant chemicals called catechins inhibit nematode hatching and also significantly inhibit nematode enzymes called proteases. The catechins affect three specific proteases that are part of a complex structure central to nematode survival. Without proper protease function, nematodes fail to develop and will die. This discovery is important because it demonstrates a molecular basis for how this plant chemical can suppress plant-parasitic nematode development and reproduction at low doses. In addition, it also demonstrates that catechins can be used as nematode control agents. This information will help scientists develop precision treatment strategies for controlling plant-parasitic nematodes and help growers seeking to decrease synthetic chemical use in crop protection.

Robotic high-throughput extraction procedures for citrus pathogens. Rapid and standardized methods are needed for detection of citrus pathogens for certification and disease management programs, including nursery pathogen-free budwood certification that is critical to establish healthy citrus orchards. ARS researchers in Parlier, California, in collaboration with scientists at the University of California, Riverside, the University of Bari in Bari, Italy, and, Consiglio Nazionale Delle Ricerche Bari (National Research Council), adapted an automated procedure for nucleic acid extraction from citrus tissue. The scientists optimized homogenization and reagent concentrations for pathogens from citrus using a robot with magnetized beads. The samples contained enough pathogen RNA and DNA to allow reliable detection of pathogens in PCR assays. The new extraction method is being used by the California Citrus Research Board's Dimitman Laboratory for diagnosis of the pathogen associated with Huanglongbing (also known as citrus greening), as well as the University of California Citrus Clonal Protection Program in Riverside, California, for citrus nursery pathogen-free budwood certification.

Chrysanthemum white rust systemically infects plants. First discovered in the United States in 1977, *Puccinia horiana* is a quarantine-significant fungal pathogen and causal agent of Chrysanthemum white rust (CWR). The pathogen was believed to have been eradicated in the United States but recently has re-appeared on several occasions in the northeast. It is important to understand how CWR infects plants and its potential to overwinter in order to implement effective control methods and eradication measures. Inoculated chrysanthemum plants were placed in a growth chamber simulating fall, winter, and spring temperatures in the northeastern United States, and newly formed stem tissues displaying CWR symptoms were examined by scanning and transmission electron microscopy by ARS scientists in Frederick and Beltsville, Maryland. Results showed *P*. *horiana* in the crown, roots, and newly developed stems and, therefore, can systemically infect chrysanthemum plants and potentially over-winter. This information is important to growers and regulatory agencies as they evaluate control measures and quarantine options.

Diagnosis of maize lethal necrosis in Kenya. Maize is a staple crop for subsistence farmers in East Africa. In 2012, a serious threat to the food security for these farmers emerged as they experienced 40 to 100 percent losses in their crops from rapidly emerging disease of corn in Kenya. Based on disease symptoms and the presence of potential insect vectors in the field, a virus was suspected as the cause of the disease. ARS researchers in Wooster, Ohio, collaborated with International Maize and Wheat Improvement Center (CIMMYT) and Kenya Agricultural Research Institute (KARI) scientists to identify two viruses, Maize chlorotic mottle virus and Sugarcane mosaic virus, in diseased maize. Together, these viruses cause maize lethal necrosis. Identification of the major pathogens involved in maize lethal necrosis allows ARS scientists and collaborators to identify disease control measures, to develop the screening protocols needed to breed disease resistant hybrids and cultivars, and to investigate the epidemiology of this rapidly spreading disease. Further, this information allows U.S. maize breeders and seed producers to stay ahead of a disease to which the $60 billion U.S. corn crop is vulnerable.

Crop genetics is a solution for acid soils and aluminum toxicity. Since 2007, ARS scientists in Pullman, Washington, have shown that low pH (of about 4) and aluminum toxicity affects over 50,000 acres of wheat in Spokane County, Washington, and Latah County, Idaho, resulting in up to 90 percent yield losses. Growers' only options have been to grow triticale or heavily lime the soil, which is not economical. ARS scientists, in collaboration with scientists at Washington State University, tested adapted wheat varieties and identified several with tolerance to aluminum and with significantly improved yields. This information was transmitted via grower talks and extension bulletins. Proper selection of tolerant varieties will allow growers to continue to produce wheat in these affected areas.

E. *typhina* infection biology. Epichloë typhina is an important fungal pathogen responsible for significant yield loss in orchardgrass (*Dactylis glomerata* L.) seed production fields. Although infections are presumed to occur through leaves and stems, details of the infection process and conditions that favor leaf infection are not well understood. Researchers in Corvallis, Oregon, determined that spore germination and growth are optimal at warm temperatures and prevented by temperatures below 41F or above 95F. They discovered that spores can survive periods of dry weather with little loss of viability, and that leaf wounds created mechanically or by an insect can stimulate growth of the pathogen within and across leaf surfaces. These results are being used to devise disease control strategies, as they indicate that the optimal time for fungicide control may be just after seed harvest, when leaf wounds, temperatures, and abundant spores are optimal for infection.

***Aspergillus flavus* whole genome sequenced**. ARS scientists in Stoneville, Mississippi, sequenced the entire genome of the fungus *Aspergillus flavus* biological control strains K49, AF36 and Afla-Guard by Illumina HiSeq sequencing, which have been assembled into about 40 million base pairs of high quality, highly overlapping sequence. These assemblies have been compared to a toxigenic strain to reveal Single Nucleotide Polymorphisms that may be useful for strain-specific identification.

Plant defense pathway in resistance to Fusarium head blight. Fusarium head blight (FHB) is one the most significant threats to wheat and barley production in the United States and worldwide; however, little is known about the genetic mechanisms that can provide resistance to this disease. Plants possess multiple genetic pathways to provide defense against pathogen attack. A novel genetic assay developed by ARS researchers in West Lafayette, Indiana, has demonstrated that one of these pathways, known as the basal defense pathway, plays an essential role in FHB resistance. Defining which defense pathway is essential for resistance to Fusarium head blight provides crucial direction for efforts to engineer wheat and barley with improved resistance.

Component 3 – Plant Health Management.

Three genes control resistance to the attack of soybean by soil-borne nematodes. ARS scientists in Madison, Wisconsin, in collaboration with university scientists, developed a quantitative assay to measure the expression of one of these genes. This gene protects soybeans from attack by the soybean cyst nematode, the most economically damaging pathogen of soybeans in the United States. Their analysis demonstrated that all three genes contribute to genetic resistance, with more copies of the gene resulting in stronger resistance. This finding opens significant new avenues of investigating how plant genes confer resistance to plant pathogens, and how plant breeders might be able to develop soybeans with stronger resistance for growers.

Classification of fusarium wilt biotypes that attack cotton. An exceptionally virulent *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) race 4 fungus was identified in cotton fields in California in 2002. Race 4 is a grave threat to U.S. cotton production because seed-cotton grown in California has been distributed across the U.S. Cotton Belt; Fov is seed transmitted, and thus U.S. fields may be infested with this pathogen. ARS scientists in College Station, Texas, investigated in detail a number of isolates of Fov and assigned them to one of three groups: 1) those capable of invading host plant xylem (water transport) cells and then spreading throughout the vascular system of the plant; 2) root-rotting pathogens; and 3) non-pathogens. This work established that earlier methods of determining Fov races and types are not reliable, and that only carefully controlled disease assays are reliable in determining virulence and thus the potential threat to cotton production. The accomplishment is foundational to ongoing research focused on protecting U.S. cotton from the devastating effects of extraordinarily virulent Fov types.

Component 4 – Alternatives to Preplant Methyl Bromide Soil Fumigation.

Anaerobic soil disinfestation as an alternative to methyl bromide fumigation. A cooperative research project between ARS in Fort Pierce, Florida, the University of Tennessee, Knoxville, and the University of California, Santa Cruz, has generated new information on Anaerobic Soil Disinfestation (ASD), a technique that utilizes the combination of a nitrogen source, such as composted broiler litter, and a carbon source, such as molasses, with soil saturation and heating to create an anaerobic condition that induces weed, nematode, and soilborne plant pathogen control. Control of yellow nutsedge emerging though the plastic early in the season with ASD was equivalent to methyl bromide in one field trial. Root-knot nematode control was influenced by initial irrigation, molasses addition, and by the incorporation of composted broiler litter. Several plant pathogenic fungi were found to be controlled by the application of litter alone. ASD was found to be more effective in the control of the strawberry charcoal rot pathogen, *Macrophomina phaseolina*, than were the commercial standard or other experimental control measures. Populations of potentially beneficial fungi, belonging to the genus Trichoderma, as well as populations of the bacteria Bacillus and Paenibacillus, were found to increase significantly with the application of ASD. Successful crop production using this method is influenced by the nematode-susceptibility of the crop**.**

Vegetable grafting for plant pathogen control. During this reporting period, field experiments were conducted and repeated in Florida in cooperation with commercial growers. A field experiment evaluating grafted heirloom tomato was performed in cooperation with an organic grower in St. Lucie County, Florida, and a sustainable grower in Palm Beach County, Florida. Also microplot, greenhouse, and growth chamber experiments were conducted to evaluate grafting techniques, virus resistance, and nematode susceptibility of rootstocks. In field trials evaluating root-stock virus resistance, heirloom tomatoes with no Tomato Yellow

Leaf Curl Virus (TYLCV) resistance produced greater yields when grafted than when non-grafted. USDA-ARS researchers are active in Executive and Advisory Team meetings and in commodity (tomato and cucurbit) Working Groups.

Organic seed treatments for organic pea production. In the Pacific Northwest, conventional pea growers use metalaxyl or mefenoxam as a seed treatment to manage Pythium seed and seedling rot; however, organic growers are not allowed to use these compounds to manage this disease. In on-farm organic farming research, organic seed treatments (biochar and cuprous oxide) were identified which, when combined with seed priming practices (pre-plant seed-soaking times in water), improved stand development and reduced disease (Pythium and Rhizoctonia seed and seedling rot) severity in certified organic pea production systems. Currently most organic pea growers do not use any seed treatments, and growers do not use priming techniques to improve plant emergence. This research determined that in replicated organic field trials, a combination of primed seed treated with cuprous oxide and biochar increased the stand development by an average of 26.5 percent and the yield by 2,071 pounds per acre over the industry standard of no seed treatment.

Sclerotinia risk management in canola. At present, about 95 percent of the U.S. canola crop is grown in North Dakota each year, and losses from Sclerotinia represent a major challenge to profitability. National Sclerotinia Initiative collaborators from North Dakota State University have developed a grower-oriented forecasting system, consisting of a general risk map and a risk calculator, designed to assist producers with managing Sclerotinia. The general risk map utilizes weather information to estimate risk of disease development throughout the canola-growing areas of the state. This map is updated twice weekly and posted on-line, beginning in mid to late June and continuing throughout the canola flowering period each season. The risk calculator combines information on cultural practices, the field-specific past history of Sclerotinia, and current weather information – retrieved from the nearest weather station – to estimate the risk of disease development. This on-line tool is currently being used by producers throughout North Dakota for the selection and timing of cultural and chemical interventions against Sclerotinia.

Developed sensitive methods to detect and quantify the corky root pathogen Sphingobium suberifaciens of lettuce. Research on *Sphingobium suberifaciens* is limited because the pathogen is not amenable to classical detection and quantification methods. An ARS researcher in Salinas, California, identified DNA sequences specific to this pathogen. The primers were used to develop pathogen specific polymerase chain reaction (PCR) and real-time PCR protocols. The protocols specifically detect and quantify the pathogen from environmental samples including lettuce roots and naturally infested field soil at levels important for disease development (200 cells per PCR sample). These methods will facilitate research on the epidemiology of the disease and allow agriculturalists to detect and quantify the pathogen before planting susceptible crops.