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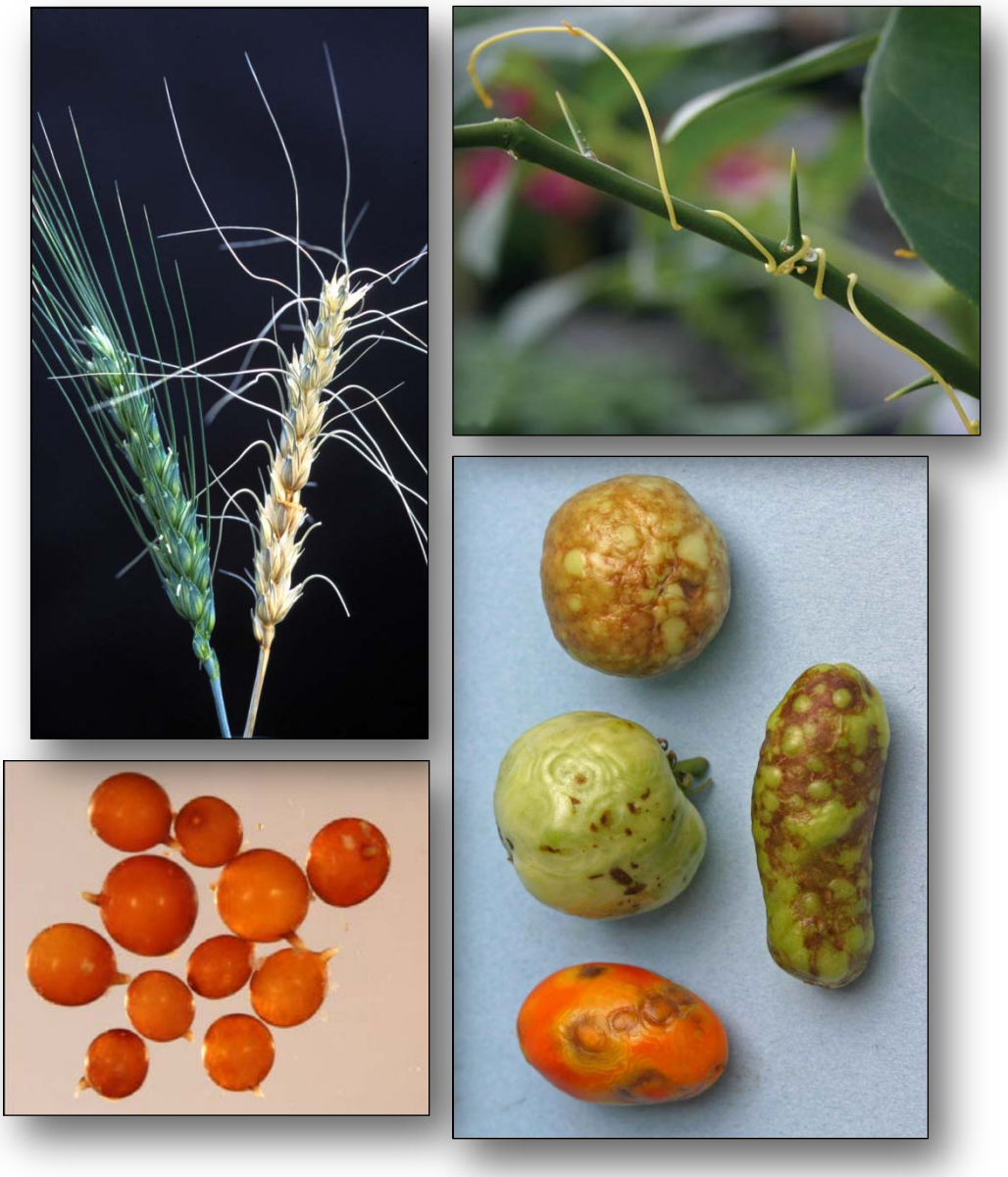
National Program 303:
Plant Diseases

Accomplishment Report 2007-2011



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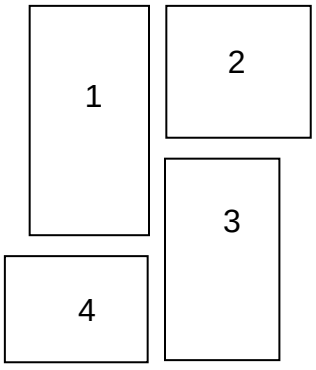

1. A healthy wheat head (left) stands in contrast to one inoculated with *Fusarium graminearum* showing severe symptoms of Fusarium head blight disease (right). *Photo by Keith Weller/ARS.*

2. Colonization of dodder by '*Candidatus* Liberibacter asiaticus' and '*Ca.* Liberibacter americanus', as featured on the cover of Phytopathology August 2010. *Photo by John Hartung/ARS.*

3. Symptoms of *Groundnut ringspot virus* on tomato fruit. ARS first reported that this virus has been found within the United States. This introduction expands the number of viruses causing disease in Florida tomatoes. *Photo by Scott Adkins/ARS.*

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National Program 303
Plant Diseases

ACCOMPLISHMENT REPORT 2007-2011

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BACKGROUND AND GENERAL INFORMATION

This report is a compilation of some of the most significant research accomplishments of the past 5 years achieved by scientists working in the USDA Agricultural Research Service's (ARS) National Program 303 (NP 303) on Plant Diseases. The NP 303 mission is to develop effective and affordable methods and strategies to reduce losses caused by plant diseases, while maintaining environmental quality. To this end, projects in this national program aim to reduce the impact that plant diseases have on decreased yields; lower product quality and shelf-life; decreased aesthetic or nutritional value; and potential contamination of food and feed with toxins. Besides the obvious monetary benefits to producers and processors, successful and consistent crop protection is a pillar of national and global food security. Additionally, the knowledge and management of plant diseases of quarantine significance are vital, not only for protecting domestic crops from foreign disease, but also for maintaining and expanding U.S. export markets for plants and plant products.

NP 303 consists of 63 active projects located in 22 different states. Most of the more than 140 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.

Management of plant diseases is essential for providing an adequate and consistent supply of food, feed, fiber, and aesthetic plants. Reducing crop losses has long been a high priority for ARS, as it has for the nation's farmers. Strategies for managing plant diseases involve a coordinated approach that includes rapid and accurate detection methods, development and use of disease-resistant varieties, and integrated management strategies that include biological controls and modified cultural practices. Developing any of these strategies depends first on identifying the pathogen causing the disease, then learning how to interrupt its disease cycle. The more that is known about the genetic, biochemical, and physiological processes operating in the host and the pathogen (and its potential vector) as infection and disease progress, the more likely a control method can be devised. Critical to developing effective control methods is an understanding of the pathogen ecology and etiology (how they survive, how they are dispersed, and how they interact with their environment), and their epidemiology (how disease epidemics develop and spread).

Figure 1: The scientists assigned to National Program 303, Plant Diseases, are conducting research in 22 states and 30 different laboratory locations.

ARS national initiatives to combat major U.S. plant disease threats are also part of NP 303. These include the U.S. Wheat and Barley Scab Initiative, the Ug99 Wheat Stem Rust Initiative, the Soybean Rust Initiative, the Sclerotinia Initiative, the Floriculture and Nursery Research Initiative, and Huanglongbing (HLB, also known as citrus greening). As such, much of the research conducted within this national program addresses the needs of industry and State and Federal regulatory agencies, including the USDA Animal and Plant Health Inspection Service (APHIS), and their efforts to prevent the spread of diseases into the United States or within the United States.

During the 5-year period covered by this report, ARS scientists developed more rapid and efficient disease diagnostic methods for streamlining and modernizing costly, time-consuming laboratory methods or for optimizing methods for field use. These new methods were then shared with Federal and State regulatory agencies, state diagnostic laboratories through the National Plant Diagnostic Network, land-grant universities, extension leaders, and agricultural technical advisors, and with breeders who used the methods in their work to develop disease-resistant varieties. ARS scientists have made significant advances in distinguishing between strains of pathogens, detecting numerous pathogens in a single test, and detecting all members of a particular family of pathogens at once. Key to these improvements is the systematics of microbes, their hosts, and vectors, where appropriate. In depth knowledge of systematics and

well curated collections have enabled ARS scientists to improve diagnostics, distinguish new pathotypes, and develop genetic markers for disease resistance development.

This report also documents advances in understanding the interactions between hosts and their pathogens, providing insight into how pathogens overcome disease resistance in crops, and other studies showing how new pathogens arise by the exchange of chromosomes. Better understanding of disease etiology and epidemiology has helped elucidate the causal agent of new and emerging diseases and the importance of specific disease vector control, such as watermelon vine decline and zebra chip of potato.

New knowledge generated by NP 303 scientists has led to environmentally safe means of controlling pathogens by applying molecular markers to improve host-plant disease resistance. Biological and cultural control strategies are also a focus area of NP 303 that benefits from research aligned with National Program 308, Methyl Bromide Alternatives, and National Program 301, Plant Genetic Resources, Genomics, and Genetic Improvement. For example, collaborations have yielded a reduction of mycotoxin contamination in corn and peanut with development of new varieties that are resistant to the causal agents and biological control agents that effectively compete with the pathogen.

PLANNING AND COORDINATION FOR THE NP 303 5-YEAR CYCLE

The current 2007-2012 Action Plan for NP 303, which served as a guide for the research within the national program, was drafted by a writing team composed of ARS scientists and members of the NP 303 National Program Leader (NPL) team in 2006. Incorporating input from customer/stakeholder interactions, the NPLs' knowledge of the science subject matter, and input from other ARS scientists, the writing team identified the priority needs that could be realistically addressed with ARS resources and base funding. These individual research needs were aggregated into problem statements under each of the four research components. The final Action Plan guided development of new individual NP 303 research projects that began the current 5-year research cycle beginning in 2007.

Next, individual project plans in NP 303 were evaluated at the beginning of the 5-year cycle (in 2007) for scientific quality and feasibility by external peer review panels. This ARS Peer Review Process, through the Office of Scientific Quality Review, is an essential part of the 5-year ARS research program cycle. This process was mandated by the Agricultural Research, Extension, and Education Reform Act of 1998, which requires successful completion of peer review as a prerequisite to performance of research. Each research project in every national program includes statements of the agricultural problem being addressed; anticipated outputs or information to be generated by the project; how the planned research contributes to mitigating or solving the larger national program problem statements; and timelines and milestones for measuring progress toward achieving project objectives. Project plans were revised in response to review panel recommendations as needed, and were implemented in 2007. The next 5-year research cycle for NP 303 will begin in 2012 with the new Action Plan that was written for 2012-2016. New plans for individual research projects are currently under formal prospective review.

Coordination and planning for NP 303 are the tasks of the NPLs who constitute the NP 303 leadership team. These NPLs also coordinate NP 303 activities with other ARS national programs and with other agencies and departments. Some of the interagency research coordination associated with NP 303 is conducted through Federal Interagency Committees such as the Invasive Terrestrial Animals and Pathogens, and the Interagency Microbial Forensics Advisory Board, as well as integrated pest management Pest Information Platform for Extension (ipmPIPE) and the National Clean Plant Network. The NP 303 NPL team also confers and coordinates with colleagues from other USDA agencies, including the U.S. Forest Service, Economic Research Service, National Institute for Food and Agriculture (NIFA), APHIS, and the Foreign Agricultural Service, as well as with the U.S. Agency for International Development (USAID) and the U.S. Department of State.

Customer and stakeholder interaction and research coordination continue to play important roles in helping NPLs guide NP 303 research to maintain its relevance to U.S. agriculture. As shown in Appendix 4 of this report, NP 303 NPLs attended or organized numerous research planning, coordination, and stakeholder workshops during 2006-2011. These workshops addressed crop/commodity-specific challenges, emerging high-priority issues, and new scientific developments in the fields, including, but not limited to pests and pathogens and their impact on crop production.

STRUCTURE OF NP 303

The NP 303 Action Plan (2007-2012) is composed of the following four components that together, and in concert with other national programs, strive to achieve breakthroughs to the understanding and control of plant diseases and to developing strategies for disease management that enhance agricultural production and value. The Action Plan is a living document that has the flexibility for change if the need arises due to significant changes in available techniques, emerging pathogens, or new information.

Component 1: Disease Diagnosis: Detection, Identification and Characterization of Plant Pathogens.

Rapid, reliable pathogen detection and identification procedures for accurate and timely disease diagnoses are of critical importance as international trade increases and as the United States and its trading partners seek to protect themselves from the introduction of exotic pathogens. Projects under this component may include research on developing or improving diagnostics for existing, emerging, or exotic pathogens; pathogen detection and/or quantification methods; systematics, evolution, and population genetics of pathogens; and understanding the etiology of exotic, emerging, and poorly understood plant diseases. Accomplishments in this component often serve the needs of Federal and State regulatory agencies and are transferred directly for use and distribution.

Component 2: Biology, Ecology, Epidemiology, and Spread of Plant Pathogens and their Relationships with Hosts and Vectors.

Critical to developing effective disease management methods is an understanding of the biology, ecology, and epidemiology of pathogens, as well as an in-depth knowledge of the fundamental biology of pathogen-host-vector interactions. This component includes

research on molecular, cellular, and organismal aspects of plant pathogens; interactions of pathogens with plant hosts and vectors; ecology, epidemiology, and spread of pathogens and vectors; and the impact of climate change on pathogens, their vectors, and disease expression.

Component 3: Plant Disease Resistance.
Host-plant resistance provides many advantages as a disease management option, including reducing or eliminating the need for pesticides. In addition, crop production in the United States is continuously threatened by the introduction of exotic plant diseases and emerging strains of domestic pathogens. To respond to these threats, scientists must anticipate and recognize plant diseases, maintaining adequate germplasm stocks from which to draw disease and vector resistance genes, and rapidly move resistance genes from unimproved germplasm into desirable cultivars. In the long term, new principles and measures for disease management will be developed for a better understanding of host-pathogen interactions. Component 3 in particular, is closely coordinated with ARS National Program 301 and university breeding programs.

Component 4: Biological and Cultural Strategies for Sustainable Disease Management.
Research conducted under this component includes manipulation of cultural practices to promote plant health or manage pathogen or vector populations; development, characterization, and deployment of biological agents that reduce pathogen or vector populations or otherwise enhance plant health; improvements to the efficacy of chemical agents to control pathogen and vector populations; and development of integrated disease management systems to improve plant health and crop production. Research under this component has yielded an array of new biocontrol agents, suppressive soils, management and cropping practices, natural products, organic amendments, and novel physical and chemical treatments.

For organizational and administrative purposes, individual NP 303 research projects are usually assigned membership to only one of these research components. However, these research categories do not act as barriers or "stovepipes" that impede research within NP 303 or across other ARS national program areas. Many individual research projects contribute to attaining the goals of several NP 303 research components or to several national programs.

RELATIONSHIP OF NP 303 TO OTHER NATIONAL PROGRAMS

ARS research is organized into four national program areas that include Nutrition, Food Safety, and Quality; Animal Production and Protection; Natural Resources and Sustainable Agricultural Systems; and Crop Production and Protection (CPP). NP 303 is one of five national programs within CPP, and research conducted under this national program often contributes to attaining the goals of other national programs:

- NP 301 – Plant Genetic Resources, Genomics and Genetic Improvement (Crop breeding, genetics, genomics, genome databases, and bioinformatics, germplasm conservation and characterization). NP 303 scientists have significant partnerships with their counterparts in NP 301 in combating plant diseases through genetic strategies for host resistance.

- NP 304 – Crop Protection and Quarantine (Insect and weed issues, trade)
- NP 305 – Crop Production (Integrated crop production systems, irrigation and spray technologies, agricultural engineering, and bees)
- NP 308 – Methyl Bromide Alternatives (Disease and pest control method alternatives to using methyl bromide)

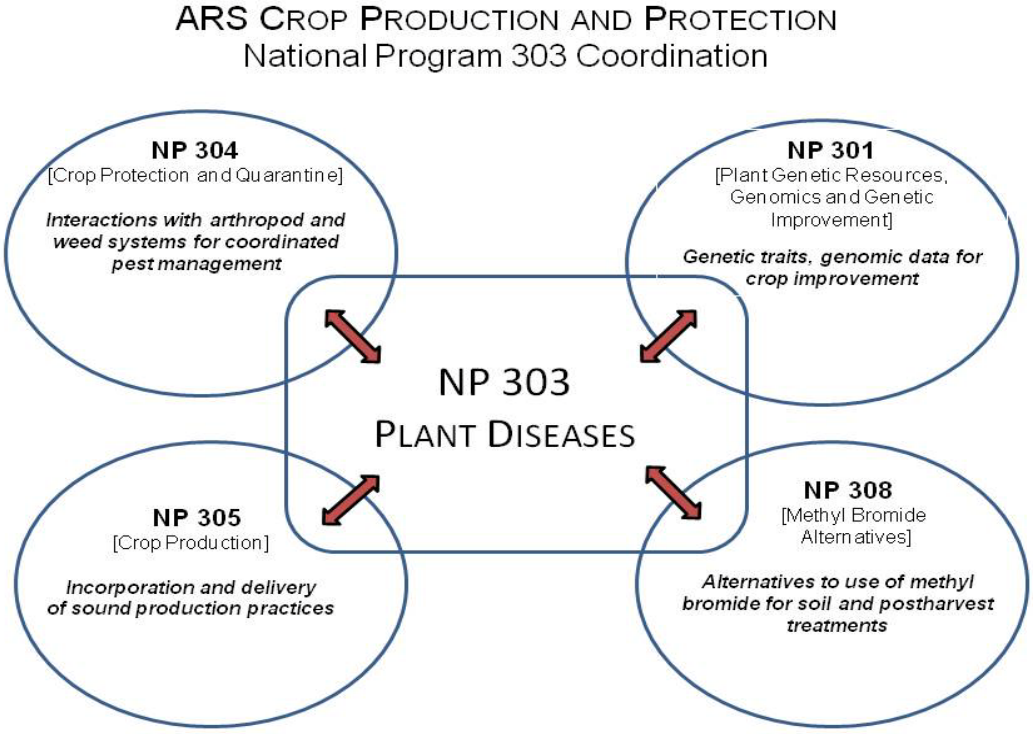

Figure 2: Research within National Program 303 is coordinated with other national programs within the ARS Crop Production and Protection section in an interdisciplinary approach to solve problems.

For example, recent ARS research on Asian soybean rust illustrates the coordination across NP 303 research components and other national programs. Researchers, extension agents, and educators across the United States formed a collaborative research effort on soybean rust prior to its entry into the United States. As part of that effort, ARS scientists with projects in Component 1 developed diagnostic assays at the ARS Fort Detrick, Maryland, facility that were then provided to APHIS for validation. These assays were used to train first detectors from diagnostic laboratories throughout the National Plant Diagnostic Network. By the time soybean rust was detected in the United States, soybean scientists and the national network of first detectors were familiar with symptoms of soybean rust and could conduct the microscopic and molecular analysis in National Plant Diagnostic Network labs in coordination with APHIS. ARS scientists with projects in Component 2 conducted research to determine the genetic variability of the pathogen, and developed methods to trap spores, with soybean and other leguminous crops serving as sentinel plants for the rest of the U.S. soybean production. They provided information that contributed to the ipmPIPE (integrated pest management, Pest Information Platform for

Extension and Education) with information from spore traps and sentinel plots delivered to the Web-based platform (www.ipmpipe.org) to track disease movement. Through national and international collaborations, ARS scientists in NP 303 and NP 301 made significant advances in identifying genetic resistance/tolerance for soybean rust in soybean germplasm, so that growers will be less likely to depend on fungicides for control. Soybean rust spore trapping methods developed by ARS scientists also were deployed in 2011 to track the movement of sugarcane orange rust (*Puccinia kuehnii*) in Florida and Louisiana cane production areas.

In an example of broader collaborations across national programs, sustainable feedstock production for biofuels has become an important focus in response to the Secretary of Agriculture's goals and priorities. Energycane (a product from sugarcane breeding programs) is considered prominently as a valuable crop for the southeastern United States as an important source of feedstocks for biofuels. To protect the U.S. sugarcane and energycane crops, and to attain the Administration's ambitious bioenergy goals (25 billion gallons of biofuel by 2025), plant diseases must be managed. Approaches in disease resistance development, along with new methods of inoculation and disease evaluations for sugarcane improvement, are an ongoing component of the sugarcane improvement for resistance to yield-limiting biotic and abiotic factors. These new approaches are being directly applied to new energycane crops as they are developed. Research in NP 303 in sugarcane pathology and disease resistance is closely tied to ARS Natural Resources and Sustainable Agricultural Systems programs that address biofuels, climate change, water efficiency, and land stewardship. In addition, there are additional examples of "cross-talk" between national program areas in Food Safety and Quality as well, including citrus-based products that may help to control citrus diseases or their vectors (volatiles for repelling the Asian citrus psyllid, processing of fruit that may be contaminated with citrus canker, juice quality as impacted by Huanglongbing), postharvest diseases, and evaluations of phenotypes toward developing resistance to toxins as food contaminants.

A public research agency, such as ARS, with a mandate for addressing national research needs has the opportunity, as well as the obligation, to engage with partners to combat threatening diseases. Advances in managing important and economically limiting diseases necessitate establishing external partnerships with public and private entities. Soybean rust, Ug99, Pierce's disease, citrus canker, and Huanglongbing (HLB; also known as citrus greening) are a few examples of significant recent threats to U.S. agriculture where NP 303 scientists took a strong interagency approach to coordinate needed research. ARS has the disease experts and longer-term base funding needed to advance national limitations in managing disease threats. Often, decades of research are available to draw from when a new disease emerges, and scientists are able to respond immediately. In the case of Pierce's disease, although at the time no resources within ARS were dedicated to this disease, researchers were redirected and existing infrastructure was made available to address research needs on all aspects of the disease, including host and vector studies. ARS worked closely with APHIS, universities, and private industry to determine the critical research needs and where to direct new funds.

Similarly, ARS partnered with APHIS and NIFA to coordinate a strong national research effort on controlling Huanglongbing. With Huanglongbing now established in all of the citrus-producing counties of Florida, and the suspected vector (the Asian citrus psyllid) increasing its

range in the United States, it was clear that despite the excellent research being conducted nationally and internationally, the incremental efforts were not well coordinated. A clear national research plan, which included technology transfer and outreach, was needed. ARS, APHIS, and NIFA joined with representatives from private industry (California, Texas, and Florida) in December 2009 to form a Science and Technology Working Group, a part of the APHIS Citrus Health Response Plan, specifically to coordinate the large body of research needed to manage citrus greening and its psyllid vector. ARS scientists in NP 303 worked as a broader team with entomology, horticulture, and genetics to identify gaps, establish priorities and develop a coordinated research plan on Huanglongbing. ARS continues to collaborate extensively with industry and Federal regulatory agencies to help in directing a national effort against Huanglongbing that includes research, but also coordinating with extension and education efforts. ARS researchers work with partners to develop research plans to manage the psyllid vector, develop therapeutic treatments for infected trees, and devise new technologies to improve host resistance to the pathogen and the insect vector. Specific advances in research on Huanglongbing within this national program are included later in this report.

The accomplishments of NP 303 were often attained in close cooperation with public and private sector collaborators, and in fact, many NP 303 researchers are co-located on land-grant university campuses. The ability of ARS to partner with land-grant universities has strengthened the capacities of both institutions. As an added benefit of this relationship with the universities, NP 303 researchers have helped to train some of the next generation of agricultural researchers. Since 2007, NP 303 scientists have directly trained and mentored 593 post-doctoral fellows and graduate and undergraduate students who have contributed significantly to the national program's research agenda. Although the training of students is not a primary focus of ARS' research program, it is nonetheless an important measure of the leadership and research community stewardship that NP 303 research leaders and scientists are contributing to agriculture and related sciences. In addition, NP 303 scientists have accommodated dozens of visiting scientists interested in specific research and collaboration, or looking to gain additional expertise in a particular aspect of research.

SCIENTIST/STUDENT TRAINING

	Visiting Scientists	Postdoctoral Fellows	Graduate Students	Undergraduate Students
Total	149	101	124	368

Table 1: Scientists and students trained by NP 303 research leaders and scientists from 2007-2011.

During the past 5 years, the progress and achievements of NP 303 were also strengthened significantly by collaborative and coordinated research supported by non-governmental organizations, private industry, and international partners. This support can be in the form of in-kind contributions, trust agreements, or cooperative research and development agreements

(CRADAs). NP 303 NPLs and scientists often participated in strategic planning and coordination efforts with these commodity research communities so as to integrate public-sector and private-sector partners into cooperative research. These partnerships enabled NP 303 to effectively leverage its resources with external resources to conduct and transfer research that addresses critical agricultural problems.

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

In this report, NP 303 accomplishments and their impact are organized and presented according to research components and their constituent problem statements as outlined in the 2007-2011 Action Plan. Under the problem statements, accomplishments are reported with specific reference to outcomes and impact. Since fiscal resources and research capacity are not uniformly distributed among the components, more accomplishments may be reported under some problem statements than others.

The content of this accomplishment report is derived from the past 5 years of NP 303 annual reports and the reports of its constituent research projects. This report stresses the impacts of those accomplishments and, where relevant, cites key publications or Web URLs documenting those accomplishments. The 63 research projects in NP 303 are listed in Appendix 1 under the associated Action Plan component and problem statement. Publications authored by NP 303 scientists are compiled in Appendix 2; and cultivars and germplasm populations developed for disease resistance and released by ARS scientists are listed in Appendix 3. Appendix 4 has a listing of new pathogens, new geographic occurrences for pathogens, and new host associations, while Appendix 5 lists the alignment of NP 303 to the ARS Strategic Plan; workshops and conferences organized by NP 303; and a chart summarizing the numbers of external grants received.

This report was prepared for an external (to USDA) retrospective review of NP 303 to assess how well this national program attained its projected goals as outlined in its current Action Plan. Accordingly, the purpose of the retrospective review is not to judge the performance of individual NP 303 research projects, but to gauge the overall impact of the national program. Consequently, the report does not attempt to catalogue all individual accomplishments of NP 303's constituent research projects. Individual scientists or projects are not identified by name in the narrative text. Instead, their achievements are described in the context of contributions towards accomplishing NP 303's stated commitments to U. S. agriculture.

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COMPONENT 1: Disease Diagnosis: Detection, Identification and Characterization of Plant Pathogens

Agricultural producers face not only a vast assortment of indigenous plant pathogens, but also many exotic organisms that could devastate U.S. agriculture if accidentally or deliberately introduced. Control or management of diseases caused by these pathogens depends upon the ability to identify the disease or the pathogen causing it. Consequently, the security of the U.S. food supply, as well as domestic and international commerce, depends in part on the ability to rapidly and accurately detect and identify plant pathogens. Further, if pathogens are new to science, knowledge of their systematic relationships to known organisms can suggest possible disease management or mitigation strategies until specific information is available for the new organism.

ARS research activities under this Component 1 include pathogen discovery, development of diagnostic methods, and pathogen systematics, and the transfer of these tools to those who will deploy them. This effort includes ARS' commitment to the documentation and preservation of biological specimens in well-curated collections. These specimens along with their documentation are important research tools serving as reference strains for systematics and diagnostics, documenting changes in pathogen populations, and for use in resistance trials for crop improvement. Outputs under Component 1 are used by researchers, by Federal and State regulatory agencies, and by disease clinics in the National Plant Diagnostic Network.

Problem Statement 1A: *New Diagnostic Methods and Tools*

Research under this problem statement is aimed at developing new diagnostic methods and tools for the reliable detection and identification of plant pathogens. ARS scientists have developed tools for rapid, accurate and sensitive methods to detect and distinguish among several pathogens simultaneously, and developed other tools that distinguish among organisms that while morphologically identical may have large differences in pathogenicity. These methods underpin the ability to manage a disease by domestic or international quarantine, and by applying other management tools in a timely manner. Many methods and keys developed by ARS are included in APHIS-approved methods lists. In addition, these same diagnostic tools facilitate research, especially in epidemiology.

The NP 303 Action Plan identified five anticipated Outputs that were expected from research addressing the needs expressed in Problem Statement 1A. The anticipated products now serve to help measure the national program's progress during the last 5 years in meeting the needs of crop researchers and producers. The list of Outputs from the Action Plan is followed by a sampling of relevant accomplishments for each Problem Statement.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. New, rapid, reliable, "field-friendly" methods with acute discriminating power for detecting and identifying plant pathogen species, strains, or pathotypes, often within

hours or minutes of specimen examination, and often using tiny amounts of plant or non-plant material.
2. Diagnostic methods capable of detecting and identifying several pathogens concurrently.
3. Inexpensive on-site or pre-plant pathogen detection methods that do not require highly trained personnel.
4. More effective methods, such as genetic marker systems and in vitro screens, for distinguishing pathogen genotypes.
5. New statistically-sound sampling methods that enable more efficient recovery/isolation of representative pathogen samples.

PROBLEM STATEMENT 1A: SELECTED ACCOMPLISHMENTS

Output 1: ***New, rapid, reliable, "field-friendly" methods with acute discriminating power for detecting and identifying plant pathogen species, strains, or pathotypes, often within hours or minutes of specimen examination, and often using tiny amounts of plant or non-plant material.***

ARS method essential for domestic and international regulation of potato cyst nematodes. Accurate identifications of deleterious nematodes recovered from plant materials introduced to or exported from the United States limit the international and interstate spread of nematodes, permitting the export of billions of dollars of agricultural products. This information is critical to the day-to-day operations of APHIS/Plant Health, Plant Protection and Quarantine (PPQ) personnel at ports-of-entry for their efforts to exclude foreign pests and detect new invasive species. ARS scientists in Beltsville, Maryland, serve as expert identifiers for unusual nematodes submitted by State and Federal regulatory agencies. These scientists also develop methods for rapid and accurate identification of nematodes, such as the method used by APHIS/PPQ for cyst nematodes that infect potato. Results of identifications by ARS Beltsville nematologists provided key information for APHIS personnel to take appropriate regulatory actions beneficial to the public. New fields in Bonneville County, Idaho, where pale potato cyst nematodes were detected were put under regulatory control and other fields were removed from regulation. Similarly, several fields once infested with the related golden cyst nematode in New York were deregulated after establishing their continued absence through observations by ARS scientists. Nematode identifications by ARS contributed to the work of 13 graduate students, and 12 visiting scientists who were trained in nematode identification. The information and identifications supplied to other users provided valuable data to research on plant-parasitic nematodes worldwide.

Skantar, A.M., Handoo, Z.A., Carta, L.K., Chitwood, D. 2007. Morphological and molecular identification of *Globodera pallida* associated with potato in Idaho. Journal of Nematology 39:133-144.

Tracking of Huanglongbing of citrus. ARS scientists in Parlier, California, and their collaborators developed high throughput, sensitive, and accurate diagnostic methods to detect the '*Candidatus* Liberibacter asiaticus' bacteria associated with the destructive disease of Huanglongbing (HLB; also known as citrus greening). These methods are currently used by the California Department of Food and Agriculture to assay for '*Ca.* Liberibacter asiaticus' directly

from the Asian citrus psyllid vector to monitor movement of the suspected pathogen as the psyllids (but not yet the bacterium) are increasingly being detected in California. Monitoring will help alert plant health officials to any introduction of the pathogen so that appropriate mitigation can be initiated. The methods are also being used by scientists developing resistant germplasm and other disease control measures.

Lin, H., Chen, C., Doddapaneni, H., Duan, Y., Civerolo, E.L., Bai, X., Zhao, X. 2010. A new diagnostic system for ultra sensitive and specific detection and quantitation of '*Candidatus* Liberibacter asiaticus', the bacterium associated with citrus Huanglongbing. Journal of Microbiological Methods 81(1):17-25.

Chrysanthemum white rust molecular diagnostic assay. Chrysanthemum white rust, caused by *Puccinia horiana*, is a foliar disease of chrysanthemums that can result in severe losses to commercial chrysanthemum production. Chrysanthemum white rust is not established in the United States, although it has been introduced and successfully eradicated. To facilitate the detection of chrysanthemum white rust in commercial nurseries, ARS scientists in Fort Detrick, Maryland developed a rapid diagnostic tool specific to *P. horiana.* The internal transcribed spacer region was cloned and sequenced from 14 isolates of *P. horiana*, and PCR primers were designed and tested for both conventional and real-time PCR assays. Both PCR assays were capable of detecting the pathogen in chrysanthemum leaf tissue prior to the onset of symptoms. This method has been used by APHIS/PPQ for detecting and identifying *P. horiana* in commercial U. S. nurseries.

Pedley, K.F. 2009. PCR-based assays for the detection of *Puccinia horiana* on chrysanthemums. Plant Disease 93:1252-1258.

Sensitive real-time polymerase chain reaction detection method for Pepino mosaic virus in tomato seeds. *Pepino mosaic virus* is a seed-borne virus disease threatening the $400 million U.S. greenhouse tomato industry. A real-time polymerase chain reaction (PCR) technique was developed by ARS scientists in Charleston, South Carolina, and their collaborators to allow sensitive and reliable detection of *Pepino mosaic virus* in tomato seed or plant tissues. Because pepino disease resistance is not available in tomato cultivars, planting certified virus-free seeds is the first step to preventing accidental introduction of this devastating virus from infected seeds to tomato production greenhouses. Currently, a commercial plant diagnostic laboratory is interested in further development of this technology into testing kits.

Ling, K., Wechter, W.P., Walcott, R.R., Keinath, A.P. 2011. Development of a real-time PCR assay for *Squash mosaic virus* useful for broad spectrum detection of various serotypes and its incorporation in a multiplex seed health assay. Journal of Phytopathology 159:649-656.

Diagnostic reagents for early detection of soybean rust. *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust, has spread from Asia to Africa, South America, and finally to North America. Since arriving in the United States in 2004, the need to use fungicide for control has increased production costs to soybean producers in the southeast United States, where the disease is most prevalent. ARS researchers in Fort Detrick, Maryland, have developed sensitive, specific antibody based diagnostic reagents that can identify and diagnose the disease in soybean leaves before symptoms occur. The diagnostic antibodies have been licensed for production of kits for

field detection of the pathogen. Early detection of soybean rust will allow soybean producers to reduce fungicide input costs leading to increased yield and profits.

Output 2: ***Diagnostic methods capable of detecting and identifying several pathogens concurrently.***

Detection of multiple pathogens. ARS scientists and their collaborators at several locations have developed methods for the simultaneous detection of multiple pathogens, particularly for pathogens of vegetatively propagated crops. These methods and reagents have been adopted by the U.S. tree crop and floriculture industries to establish effective virus testing protocols to improve clean stock production for vegetatively propagated annuals and perennials. Some of the significant accomplishments and their initial impact follow:

Clean citrus budwood. Most citrus nurseries in central California maintain registered scion trees and budwood increase blocks in open fields, and they also grow citrus propagations in the field. All nursery trees must be free of *Citrus tristeza virus* and, because of the spread of the virus, nursery trees must be tested to insure they are virus-free. ARS researchers in Parlier, California, tested commercial monoclonal antibodies as cocktails for universal detection of California *Citrus tristeza virus* strains. Two commercial nurseries are now using a *Citrus tristeza virus* immunoprint test kit assembled by the scientists to test all citrus for the virus, as is the University of California Lindcove Research and Extension Center in Exeter, California. *Citrus tristeza virus*-infected trees identified by the tissue-print method are removed immediately in this program, thus preventing additional dissemination of the pathogen.

Vidal, E., Yokomi, R.K., Moreno, A., Bertolini, E., Cambra, M. 2011. Calculation of diagnostic parameters of advanced serological and molecular tissue-print methods for detection of *Citrus tristeza virus*. A model for other plant pathogens. Phytopathology 104:114-121.

Development of a polyprobe to detect six viroids of pome and stone fruit trees. Ensuring that plants leaving quarantine are pathogen-free is a vital step in preventing pathogen spread. Rapid and accurate testing for diseases in quarantine can shorten quarantine duration, saving time and money. Six viroids, all important quarantine pathogens in the international movement of germplasm, have been reported as infecting pome and stone fruit trees. ARS scientists in Beltsville, Maryland, developed an assay using a non-radioactive probe (polyprobe) to detect all six viroids on a single membrane using dot blot hybridizations. The polyprobe is highly sensitive in detecting the viroids in trees with both single and mixed infections. The assay was validated by testing hundreds of field samples, including samples from the National Plant Germplasm System pome and stone fruit collections. The technology has been transferred to APHIS for possible inclusion in quarantine testing protocols. Two material transfer agreements were established and these culminated in a biological licensing agreement with a commercial diagnostic company for a detection tool for viroids that infect pome and stone fruit trees (USDA License # 1555).

Liming, L., Li, R., Mock, R.G., Kinard, G.R. 2011. Development of a polyprobe to detect six viroids of pome and stone fruits. Journal of Virological Methods171:91-97.

Universal plant microarray for virus detection. As complete sequences of viral species become available, and bioinformatics software becomes more advanced, it is becoming easier to identify regions of sequence that are highly conserved among isolates of an individual viral species and that can be developed as probes for reliable detection of all isolates. ARS scientists in Beltsville, Maryland, developed a microarray to detect viruses in fruit trees. By relying on unbiased amplification techniques rather than primers specific to each virus or taxonomic group, microarrays offer an alternative approach to the challenge of screening a single sample for multiple viruses by multiplexing broad spectrum PCR assays. Such a microarray would be able to identify previously unrecognized viruses as members of the characterized virus genera. This ability is of great value to quarantine and clean stock programs where interception of unknowns is of paramount importance.

Hammond, J. 2011. Universal plant virus microarrays, broad spectrum PCR assays, and other tools for virus detection and identification. Acta Horticulture Proceedings 901:49-60.

Output 3: ***Inexpensive on-site or pre-plant pathogen detection methods that do not require highly trained personnel.***

Some disease management options, such as soil treatments, can only be applied prior to planting. Thus, detection of pathogens prior to disease development, or even prior to planting, provides important information when optimizing disease management. These data are often integrated into disease forecasting models from which grower alerts are issued. Key accomplishments follow:

Rust spore detection. ARS scientists in St. Paul, Minnesota, collaborated with the National Atmospheric Deposition Program Development (NADP), an organization overseeing the long-term sampling and analysis of precipitation in the United States, to develop a national monitoring system based on detection of fungal spores in rain. This system provided an early warning system for the spread of the Asian soybean rust pathogen. Weekly precipitation samples from NADP sampling locations were tested using a DNA-based assay (PCR) to map, and then to predict, the deposition of *Puccinia pachyrhizi* spores in the U.S. soybean growing regions. ARS scientists in St. Paul with ARS scientists in Canal Point, Florida, and Beltsville, Maryland, used this surveillance method to detect the sugarcane orange rust pathogen and the wheat stem rust pathogen (www.ars.usda.gov/Main/docs.htm?docid=14574), respectively. Scientists and agricultural specialists are using data from this monitoring system to improve field scouting programs, develop disease management recommendations for farmers, and monitor the movement of other plant pathogens dispersed in the atmosphere.

Isard, S., Barnes, C., Hambleton, S., Ariatti, A., Russo, J., Tenuta, A., Gay, D., Szabo, L.J. 2011. Predicting soybean rust incursions into the North American continental interior using crop monitoring, spore trapping, and aerobiological modeling. Plant Disease 95(11):1346.

Glynn, N.C., Dixon, L.J., Castlebury, L.A., Szabo, L. J., Comstock, J.C. 2010. PCR assays for the sugarcane rust pathogens *Puccinia kuehnii* and *P. melanocephala* and detection of a SNP associated with geographical distribution in *P. kuehnii*. Plant Pathology 59:703-711.

Barnes, C.W., Szabo, L.J., Bowersox, V.C. 2009. Detection of *Phakopsora pachyrhizi* spores in rain using a real-time PCR assay. Phytopathology 99:328-338.

Powdery mildew detection and fungicide model. To improve early detection of grape powdery mildew, ARS researchers in Corvallis, Oregon, developed a quantitative PCR assay and inexpensive spore traps shown to be reliable under commercial conditions for the detection of airborne spores of grape powdery mildew. The team demonstrated the commercial feasibility of using PCR detection of airborne spores of this pathogen to initiate fungicide applications to control the disease. Three years of field validation in as many as 10 commercial vineyards per year demonstrated that the assay helped growers avoid 2.3 fungicide applications per year, saving more than $113 an acre in application costs without increasing disease development. This finding demonstrates that withholding fungicide applications until the pathogen is detected in the air is viable commercially. Commercial application of these procedures has the potential to significantly reduce fungicide use and associated application costs for managing powdery mildew.

Pfender, W. F., Gent, D. H., Mahaffee, W. F., Coop, L. B., and Fox, A. D. 2011. Decision aids for multiple-decision disease management as affected by weather input errors. Phytopathology 101:644-653.

Output 4: ***More effective methods, such as genetic marker systems and in vitro screens, for distinguishing pathogen genotypes.***

Differentiation of citrus tristeza virus strains. One strain of a plant pathogen may produce severe disease while a morphologically identical organism may produce mild disease or no disease at all. The ability to distinguish among strains without the costly and time-consuming pathogenicity studies and host range inoculations has obvious economic advantages. ARS scientists in Parlier, California, and their collaborators developed multiplex real-time PCR assays to differentiate strains of *Citrus tristeza virus* between economically important (virulent stem-pitting and seedling yellows) versus non-economic (mild in sweet orange/sour orange on tolerant or resistant rootstock). The grower-funded Citrus Pest Detection Program, based in Tulare, California, has adopted this system to identify virulent strains of the virus and to promptly remove infected plants as a control measure for *Citrus tristeza virus* in the San Joaquin Valley. Prior to development of the assay, the ARS researchers also established the genetic diversity of *Citrus tristeza virus* in the San Joaquin Valley.

Yokomi, R.K., Saponari, M. 2011. Molecular analysis among MCA13-reactive isolates reveals a rapid strategy for assessment of Citrus tristeza virus severity. Acta Horticulturae 892:251-256.

Discriminating Macrophomina strains. In both the United States and Israel, disease caused by *Macrophomina phaseolina* has increased on strawberry as alternatives to soil fumigation with methyl bromide have been implemented. ARS scientists in Jackson, Tennessee, developed 27 monomorphic markers from *M. phaseolina* that rapidly identify this species in clinical

specimens. While previously available polymorphic markers can be used for population genetics studies of *M. phaseolina*, the group of SSR markers developed by ARS researchers enriched the limited molecular marker resource known for *M. phaseolina.* The markers are currently facilitating a large scale screening for *Macrophomina* on strawberry in California where this pathogen is becoming a serious problem.

Arias De Ares, R.S., Ray, J.D., Mengistu, A., Scheffler, B.E. 2011. Discriminating microsatellites from *Macrophomina phaseolina* and their potential association to biological functions. Plant Pathology 60:709-718

Output 5: ***New statistically-sound sampling methods that enable more efficient recovery/isolation of representative pathogen samples.***

Due to the significant overlap in research products and to avoid duplication, the accomplishments contributing to Output 5 have been combined with accomplishments in Component 2C, Output 1: *Robust statistical models to quantify relationships between disease levels and economic loss and analyzing impact to disease.*

Problem Statement 1B: ***Detection, Identification, Characterization, and Classification of Pathogens***

Research under Problem Statement 1B focuses on systematics, characterization, and phylogeny of plant pathogens, as well as curation of the databases and physical specimens associated with these activities. It also includes the description of pathogens, or new pathogen strains, new to science, with new host associations, or newly recognized geographical occurrence.

ARS scientists have discovered and characterized pathogens new to science, and new pathogen host associations or distributions. They developed robust taxonomic and phylogentic tools that enhance understanding of pathogen relationships, and they curated large, publically-available collections of pathogens.

The NP 303 Action Plan identified seven anticipated Outputs that were expected to result from research addressing the needs expressed in Problem Statement 1B. Accomplishments that illustrate those products for this Problem Statement follow the list of Outputs.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. Discovery of plant diseases and pathogen species and strains new to science or new to the United States, together with assignment of formal scientific names for the new species or variants.
2. Detection and identification of pathogens known to cause or potentially cause U.S. agricultural losses.
3. Characterization of the key genetic and biological features of exotic plant pathogens in advance of their introduction into the United States.

4. Systematically valid, accurate, and comprehensive phylogenetic systems for classifying and understanding pathogen evolutionary relationships that are linked to, and integrated with, voucher specimen collections and databases.
5. Diagnostic keys, compendia, and other guides for identifying pathogens and diseases.
6. More accurate and comprehensive phylogenetic classifications that can predict agriculturally relevant aspects of pathogen biology.
7. Enhanced knowledge of pathogen genetic diversity, especially with respect to pathogenicity and evolution.

PROBLEM STATEMENT 1B: SELECTED ACCOMPLISHMENTS

The accomplishments for the two following anticipated Outputs are so closely integrated that they are presented together to avoid duplication.

Output 1: ***Discovery of plant diseases and pathogen species and strains new to science or new to the United States, together with assignment of formal scientific names for the new species or variants.***
Output 2: ***Detection and identification of pathogens known to cause or potentially cause U.S. agricultural losses.***

Recognition of new pathogens is the first step towards management of diseases caused by the pathogens. Recognition of a known pathogen on a new host may indicate a new strain of the pathogen, and can provide information about hosts that may serve as reservoirs for the pathogen. In addition, reports of pathogens in new geographical locations can indicate the spread of a pathogen, which can be particularly important for pathogens not previously reported in the United States.

Over the past 5 years, ARS scientists have been prolific in identifying new pathogens and pests and new hosts of previously known pathogens, including the first report of the alternate host (Berberis) of the wheat stripe rust pathogen.

Given the number of new viruses, fungi, nematodes, bacteria, etc., discovered, named, and reported by ARS scientists, it would not be feasible to list them all here. However, the citations of publications regarding these new reports are compiled in Appendix 4. The following is a brief summation of what ARS scientists have found (each new report counted in one category only):

New pathogens:

- 10 new fungal species and one new fungal genus;
- 10 new phytoplasmas, including one new group and two new subgroups;
- Four new nematode species and a new nematode genus;
- Two new viruses; and
- New strains of these pathogens, and biocontrol fungi and bacteria.

New hosts were found for:

- 11 bacterial diseases; 10 fungal diseases; nine phytoplasma diseases; seven virus diseases; three viroid diseases; and one nematode.

Pathogens new to the United States:

- Seven fungal pathogens; two viruses; one nematode; one viroid; and one strain of *Phytophthora ramorum.*

Pathogens found in new geographic locations within the United States:

- Eight viruses; seven fungal pathogens; two nematodes; and one viroid.

Below a select few of these discoveries are described in greater detail:

Two new bunt fungi. A volunteer rye plant (*Secale cereale*) infected by a reticulately spored species of Tilletia was collected in a wheat field in southeastern Idaho and was tentatively identified as *Tilletia contraversa* (dwarf bunt pathogen of wheat) by ARS scientists in Beltsville, Maryland. A phylogenetic analysis demonstrated that the rye-infecting bunt was distinct from *T. contraversa* and also from the common bunt pathogens of wheat bunt, *T. caries* and *T. laevis*. This new rye-infecting bunt fits within the species concept of *T. secalis*, a pathogen of cultivated rye in Europe. The ability of *T. contraversa, T. caries,* and *T. laevis* to infect rye, and of *T. secalis* to infect wheat has resulted in confusion over the identity of bunts infecting rye. This study is the first demonstration that *T. secalis* of rye is genetically distinct from the wheat bunt pathogens, and the first report of *T. secalis* in North America. These scientists also discovered a new species of the bunt fungus, *Tilletia puccinelliae*, important to the grass seed trade, especially when inspecting grass seeds for import or export

Bao, X., Carris, L.M., Huang, G., Luo, J., Liu, Y., Castlebury, L.A. 2010. *Tilletia puccinelliae*, a new species of reticulate-spored bunt fungus infecting *Puccinellia distans*. Mycologia 102:613-623.

Carris, L.M., Castlebury, L.A. 2008. The first report of the rye smut, *Tilletia secalis*, from North America. North American Fungi 3(7):147-159.

Orange rust of sugarcane. Reports from growers of unusual rust symptoms in the south Florida sugarcane growing area were investigated during the spring of 2007. ARS scientists in Canal Point, Florida, and Beltsville, Maryland, identified the symptoms as sugarcane orange rust caused by *Puccinia kuehnii,* the first reported incidence of this disease in the Western Hemisphere. These scientists are continuing to develop new high-yielding sugarcane cultivars resistant to orange rust, as well as other major diseases, including brown rust. Orange rust adds complexity to the sugarcane breeding and disease management program because it extends the season of rust incidence beyond that of brown rust alone. Orange rust has not yet been found outside Florida. In related research, ARS scientists compared orange rust of sugarcane to the common brown rust (*Puccinia melanocephala*) on sugarcane using specimens from throughout the world. Using DNA sequence data, they determined that the two rust species are not closely related, despite infecting the same host.

Comstock, J.C., Sood, S.G., Glynn, N.C., Shine, Jr., J.M., Mckemy, J.M., Castlebury, L.A. 2008. First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in the United States and Western Hemisphere. Plant Disease 92:175.

Triticum mosaic virus. *Triticum mosaic virus*, a recently discovered wheat virus in Nebraska, was genetically characterized by ARS scientists in Lincoln, Nebraska. Based on these findings, the International Committee on Taxonomy of Viruses established a new plant virus genus (Poacevirus) in the family Potyviridae with *Triticum mosaic virus* as the type species.

Tatineni, S., Ziems, A. D., Wegulo, S. N., French, R. 2009. Triticum mosaic virus: A distinct member of the family Potyviridae with an unusually long leader sequence. Phytopathology 99:943-950.

Output 3: ***Characterization of the key genetic and biological features of exotic plant pathogens in advance of their introduction into the United States.***

In addition to discovering and characterizing new plant pathogenic species abroad, ARS scientists and their collaborators discovered key information about pathogens of high consequence that are not yet in the United States through individual or larger collaborative research projects.

Characterization of exotic plant pathogens. ARS scientists in Beltsville, Maryland developed and maintain a list of "100 Fungi on Important Agricultural and Forest Crops Not in the United States" that can be accessed through the drop down menu at http://nt.ars-grin.gov/sbmlweb/fungi/diagnosticfactsheets.cfm . The list includes diagnostic and taxonomic information. Similarly, ARS scientists provide diagnostic and taxonomic information on "Invasive and Emerging Fungi in the United States" at http://nt.ars-grin.gov/sbmlweb/fungi/diagnosticfactsheets.cfm. Both lists provide alerts of potential dangers to U.S. crop production.

Wheat stem rust. New, highly virulent races of the wheat stem-rust pathogen *Puccinia graminis* f. sp. *tritici* that recently arose in east Africa are of great concern due to their ability to overcome most known host genes for resistance, though these strains are not yet in the United States. ARS scientists sequenced the genome of the stem rust pathogen (See Component 2) and determined the lineage of this strain. The scientists determined that the Ug99 strain is genetically distinct from common North American strains and that the Ug99 family contains at least five members. More than 1 million SNPs of the pathogen genome have been identified. This information is being used to develop strain-specific diagnostics for this important pathogen, as well as by plant breeders developing cultivars resistant to these strains of the pathogen (See Component 3).

Jin, Y., Szabo, L.J., Pretorius, Z. 2008. Virulence evolution within the Ug99 lineage. http://hdl.handle.net/2123/3435.

Differentiation of biovars of Ralstonia solanacearum. *Ralstonia solanacearum*, which attacks over 450 plant species, is generally classified into 5 races based on host range and

5 biovars based on carbohydrate utilization. *R. solanacearum* race 3, biovar 2 is very destructive and causes brown rot on potato, and is a quarantined and select agent pathogen in the United States. ARS scientists in Beltsville, Maryland and their university collaborators improved the biovar test by using phenol red as a pH indicator to produce an unambiguous color change at a higher pH that also uses one-tenth the amount of media and reagents. The newly-developed test takes 2 days to resolve biovar differences, compared to several weeks for the standard test. As part of their research, these scientists also determined the genetic diversity and host range of domestic isolates of *R. solanacearum* and devised management strategies in preparation for the potential introduction of the new aggressive form of the pathogen found abroad. The improved biovar differentiation test will help State and Federal regulatory officials make timely decisions to prevent and exclude the brown rot pathogen from becoming established in the United States. These scientists also determined the genetic diversity and host range of domestic isolates of *R. solanacearum* and devised management strategies in preparation for the potential introduction of the new aggressive form of the pathogen found abroad.

Norman, D.J., Huang, Q., Yuen, J.M.F., Mangravita-Novo, A., Byrne, D. 2009. Susceptibility of Geranium cultivars to *Ralstonia solanacearum*. HortScience 44(5):1-5.

Norman, D.J., Zapata, M., Gabriel, D.W., Duan, Y., Yuen, J.F., Mangravita-Novo, A., Donahoo, R. 2009. Genetic diversity and host range variation of *Ralstonia solanacearum* strains entering the United States. Phytopathology 99:1070-1077.

Output 4: ***Systematically valid, accurate, and comprehensive phylogenetic systems for classifying and understanding pathogen evolutionary relationships that are linked to, and integrated with, voucher specimen collections and databases.***

ARS scientists in Beltsville, Maryland and their collaborators developed robust phylogentic systems on information closely tied to voucher specimens for all groups of plant pathogens. These Web sites, databases, and physical specimens are vital research resources for scientists around the world. Some examples include:

U. S. National Fungus Collections. The U. S. National Fungus Collections (www.ars.usda.gov/Services/docs.htm?docid=9397) have grown from less than 3,000 specimens in 1885 to more than 1.1 million specimens in 125,000 taxa that are curated today by ARS, including 27,000 type specimens. Specimens are publically available to recognized institutions and individuals. Additional supporting information is provided by the National Fungus Collections library of 56,000 volumes and 75,000 pamphlets and reprints. Many historical sources, such as Saccardo's Sylloge Fungorum, are digitally searchable through this Web site.

Keys and other means of identifying fungi, as well as mycological descriptions, are available at http://nt.ars-grin.gov/fungaldatabases/specimens/specimens.cfm. This database evolved from the ARS Index of Plant Diseases of the United States (1960), which was updated as separate reference books in the 1980s. In addition to being more

comprehensive than the original host index, the database is easily searchable on any field, and is linked to pathogen and disease information. The databases further provide links to literature references, and increasingly to links to macroscopic and microscopic images of specimens.

Fungal phylogenetic relationships. ARS scientists continue to delineate and clarify phylogenetic relationships in fungal taxa ranging from species complexes to the Division/Phylum level (Ascomycota), and this information is associated with the physical specimens in the collections.

Hirooka, Y., Rossman, A.Y., Chaverri, P. 2011. Morphological and phylogenetic analyses of the *Nectria cinnabarina* species complex. Studies in Mycology 68:35-56.

Mejia, L., Rossman, A.Y., Castlebury, L.A., White, J. 2011. A systematic account of the genus Plagiostoma (Gnomoniaceae, Diaporthales) based on morphology, host-associations, and a four gene phylogeny. Studies in Mycology 68:211-235.

Schoch, C., Blackwell, M., Bonito, G., Castlebury, L.A., Crous, P., Geiser, D., Lutzoni, F., O Donnell, K., Rossman, A.Y., Spatafora, J. 2009. The Ascomycota tree of life: A phylum wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Systematic Biology 58(2):224-239. http://dx.doi.org/10.1093/sysbio/syp020.

Walker, D., Castlebury, L.A., Rossman, A.Y., Sogonov, M., White, J. 2010. Systematics of the genus Gnomoniopsis (Gnomoniaceae, Diaporthales) based on a three gene phylogeny, host associations, and morphology. Mycologia 102:1479-1496.

Nematode collection and phylogenics. The USDA Nematode Collection and Database http://nt.ars-grin.gov/nematodes/search.cfm maintained by ARS researchers, is the most important repository of nematode reference specimens and data in the world. In the past 5 years, ARS scientists have added thousands of slides, vials, and related information from worldwide sources (now about 48,000 slides and vials and 38,000 records), and loaned hundreds of specimens to nematode taxonomic specialists, other scientists, and students, and to commercial and State government identifiers of field samples throughout the world. The accurate identifications facilitated by the collection have resulted in science-based decisions made by regulatory and other personnel. Access to this Web site has steadily increased to a nearly 39,000 times over the past 5 years, including at least 10,000 times in 2011.

Handoo, Z.A., Iqbal E.Y., Kazi, N., Shahina, F. 2010. Two new species of Paurodontella Husain and Khan, 1968 (Nematoda: Sphaerulariidae) associated with wheat and a diagnostic compendium to the genus. Nematology 12(2):181-192.

Skantar, A.M., Handoo, Z.A., Carta, L.K., Zasada, I. A., Ingham, R.E., Chitwood, D.J. 2011. Morphological and molecular characterization of Globodera populations from Oregon and Idaho. Phytopathology. 101(4): 480-491.

Van Den Berg, E., Subbotin, S.A., Handoo, Z.A., Tiedt, L.R. 2009. *Hirschmanniella kwazuna* sp. n. from South Africa with notes on a new record of *H. spinicaudata*, Schuurmans Stekhoven,1944, Luc & Goodey, 1964 (Nematoda: Pratylenchidae)

and on the molecular phylogeny of *Hirschmanniella* Luc & Goodey, 1964. Nematology 11(4):523-540.

Phytoplasma phylogenetics. ARS is a world leader in phytoplasma discovery and phytlogenetics, and has developed a publically available classification tool for phytoplasmas. By revealing new plant diseases and previously unknown phytoplasma lineages, this work impacts plant pathological research, aids in designing new technologies to detect phytoplasmas, and enables development of strategies to prevent spread of the phytoplasma. Tools developed for identification of phytoplasmas are employed by APHIS in regulation of plant materials.
http://plantpathology.ba.ars.usda.gov/phytoplasma.html

Zhao, Y., Wei, W., Lee, I., Shao, J.Y., Suo, X., Davis, R.E. 2009. Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). International Journal of Systematic and Evolutionary Microbiology 59(10):2582-2593.

Output 5: ***Diagnostic keys, compendia, and other guides for identifying pathogens and diseases.***

During the past 5 years, ARS scientists and their collaborators have developed keys and compendia for morphological identification of pathogens and diseases as products of this Output. They contributed to most American Phytopathological Society disease compendia and served as editors for compendia on diseases of beet, chickpea, hops, peanut, soybean, and strawberry. Also, ARS scientists and collaborators have developed tools for identification of pathogens in specific taxa.

For example, ARS scientists in St. Paul, Minnesota, manage an internationally recognized Web site on cereal rust disease (www.ars.usda.gov/Main/docs.htm?docid=9854). This site provides the most comprehensive source of information on the current and past cereal rust disease development in the United States, while facilitating access to cooperators around the country. Additionally, the site provides fundamental information on the cereal rusts, cereal rust resistance genes, germplasm evaluations, cereal rust resistance gene postulations, annual cereal rust race surveys, and other information necessary to help minimize the impact of cereal rusts. The Web site has received more than 430,000 hits since 2007.

Additional examples of identification tools developed and maintained by ARS include the interactive websites:

- Tilletia identification: http://nt.ars-grin.gov/taxadescriptions/tilletia/Index2.cfm;
- Legume rust identification: http://nt.ars-grin.gov/taxadescriptions/keys/LegumeRustsIndex.cfm;
- Ravenelia (rust) identification: http://nt.ars-grin.gov/taxadescriptions/keys/RaveneliaIndex.cfm; and
- Trichoderma identification: http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm.

De Respinis, S., Vogel, G., Benagli, C., Tonolla, M., Petrini, O., Samuels, G.J. 2010. MALDI-TOF MS of Trichoderma: A model system for the identification of microfungi. Mycological Progress. 9(1):79-100.

Database contributions. ARS scientists also contribute significantly to other databases for pathogen identification not maintained by ARS, including Phytophthora-ID: http://phytophthora-id.org/ for the identification of Phytophthora species; and the *Phytophthora ramorum* multilocus genotyping database: http://oregonstate.edu/~grunwaln/index.htm .

Grunwald, N.J., Martin, F.N., Larsen, M.M., Sullivan, C.M., Press, C.M., Coffey, M.D., Hansen Everett, M., Parke, J.L. 2011. Phytophthora-ID.org: a sequence-based Phytophthora identification tool. Plant Disease 95(3):337-342.

Output 6: ***More accurate and comprehensive phylogenetic classifications that can predict agriculturally relevant aspects of pathogen biology.***

Comprehensive fungal phylogenetic analyses. ARS scientists in Beltsville, Maryland, and their collaborators published an in-depth phylogenetic analysis of the fungal order Diaporthales. This important taxon contains the chestnut blight, apple scab, and wheat glume blotch pathogens, including a key to 59 species. Monographic accounts of three canker-causing genera in the Diaporthales were also published. Each monograph included descriptions and illustrations, a key to species, and a multi-gene phylogeny to show relationships within the genus. Phylogenetic relationships in the Nectria complex were determined using a multi-gene approach and elucidated relationships in host-pathogen specificity. Each of 56 species in the three genera was described, with illustrations and a key for identification.

Hirooka, Y., Rossman, A.Y., Chaverri, P. 2011. Morphological and phylogenetic analyses of the *Nectria cinnabarina* species complex. Studies in Mycology68:35-56.

Schoch, C., Blackwell, M., Bonito, G., Castlebury, L.A., Crous, P., Geiser, D., Lutzoni, F., O Donnell, K., Rossman, A.Y., Spatafora, J. 2009. The Ascomycota tree of life: A phylum wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Systematic Biology 58(2):224-239. http://dx.doi.org/10.1093/sysbio/syp020.

Schoch, C., Crous, P., Groenewald, J., Barres, B., Boehm, E., Degruyter, J., De Hoog, G., Dixon, L.J., Fournier, J., Grube, M., Gueidan, C., Harada, Y., Hatakeyama, Hirayama, K., Hosoya, T., Hyde, K., Jones, E., Kohlmeyer, J., Lucking, R., Lumbsch, H., Lutzoni, F., Marvanova, L., Mbatchou, J., Miller, A., Mugambi, G., Muggia, L., Nelson, M., Nelson, P., Owensby, C., Phongpaichit, S., Pointing, S., Pujade-Renaud, V., Raja, H., Rivas Plata, E., Robbertse, B., Ruibal, C., Sakayaroj, J., Sano, T., Selbmann, L., Shearer, C., Shirouzu, T., Slippers, B., Suetrong, S., Tanaka, K., Volkmann-Kohlmeyer, B., Wood, A., Woudenberg, J., Yonezawa, H., Zhang, Y., Spatafora, J. 2009. A class-wide phylogenetic assessment of Dothideomycetes. Studies in Mycology 64:1-15.

Output 7: ***Enhanced knowledge of pathogen genetic diversity, especially with respect to pathogenicity and evolution.***

Phytopthora ramorum lineage. ARS scientists in Corvallis, Oregon, and their university collaborators determined relationships among strains of the Sudden Oak Death pathogen, *Phytophthora ramorum,* and determined that the pathogen may have been introduced into the United States three times. This genetic information has been used by APHIS in its discussion with U.S. trading partners about the possible movement of this pathogen through international trade. [See also Database Contributions in Output 5 above.]

Elliot, M., Sumampong, G., Varga, A., Shamoun, S.F., James, D., Masri, S., Grunwald, N.J. 2011. Phenotypic differences among three clonal lineages of *Phytophthora ramorum*. Forest Pathology 41:7-14.

Goss, E.M., Larsen, M.M., Vercauteren, A., Werres, S., Heungens, K., Grunwald, N.J. 2011. *Phytophthora ramorum* detections in Canada: evidence for migration within North America and from Europe. Phytopathology 101:166-171.

Phytoplasma evolution. Using their previous discovery of unique phytoplasmal genome architecture – structures named sequence-variable mosaics (SVMs), ARS scientists in Beltsville, Maryland, and their collaborators found evidence for the role of mobile genetic elements in the evolution of phytoplasmas. They found evidence for SVMs in phytoplasmas representing diverse branches of the phytoplasma evolutionary tree, placing SVM formation at the root of phytoplasma evolution. They postulated that the size and nonrandom chromosomal distribution of SVMs could be explained by recurrent, mobile element attack targeted to specific regions in the phytoplasma chromosome and that hyper-variable regions within the SVMs remained active as sites for horizontal acquisition of foreign genes. Because no SVM-like structures could be identified in genomes of ancestral relatives, including *Acholeplasma* spp., they hypothesize that ancient phage attacks leading to SVM formation occurred after divergence of phytoplasmas from acholeplasmas, triggering evolution of the phytoplasma clade.

Wei, W., Davis, R.E., Jomantiene, R., Zhao, Y. Ancient, recurrent phage attacks and recombination shaped dynamic sequence-variable mosaics at the root of phytoplasma genome evolution. Proc. Natl. Acad. Sci. U S A 2008 August 19; 105(33): 11827–11832.

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COMPONENT 2: Biology, Ecology, Epidemiology, and Spread of Plant Pathogens and Their Relationships with Hosts and Vectors

Basic biology, molecular genetics, and epidemiology of plant pathogens and the diseases they cause are key for devising means of controlling plant diseases and limiting their effect on crop losses. This often entails molecular aspects of host-pathogen-vector interactions that provide new targets for interruption of the disease cycle. Knowledge of basic biology and etiology of a disease are also a necessary area of study and is essential for solving agricultural problems that require an integrated approach. Genetic and genomic information made available through advancing technologies has provided insight into disease complexes caused by plant pathogenic fungi, bacteria, viruses, and nematodes. Research conducted by ARS scientists in Component 2 provides valuable information to contribute to other Component areas in NP 303, from improved diagnostics and systematics to new approaches for disease resistance and crop improvement.

PROBLEM STATEMENT 2A: *Pathogen Biology, Virulence determinants, and Genetics of the Pathogen*

As molecular technologies improve in time and cost required, pathogen sequences are more accessible to better understand host-pathogen relationships and the function of genes related to pathogenicity and other important traits, and aid in the development of diagnostic tools. For pathogens that are to date unculturable, clues can be acquired through sequences that may aid growing these fastidious organisms in vitro. This is especially relevant to the Liberibacter species associated with Huanglongbing (HLB, also known as citrus greening), zebra chip of potato, and "tomato psyllid yellows." Pathogen sequences and their representative isolates and/or cultures are important research tools, serving as reference strains for systematics and diagnostics, and for use in resistance trials for crop improvement.

ARS scientists have been instrumental in advancing new molecular technologies that have lead to the discoveries of the molecular basis of pathogenicity, novel means of pathogen control, and host resistance response. Fundamental discoveries were also made regarding the structure and function of pathogen genomes and evolutionary relationships among pathogens.

The NP 303 Action Plan identified five anticipated Outputs that were expected to result from research addressing the needs expressed in Problem Statement 2A. The anticipated products now serve to help measure the national program's progress during the last 5 years in meeting the needs of crop researchers and producers. Accomplishments that illustrate those products for this problem statement follow the list of Outputs.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. Description of pathogen genomes, development of pathogen libraries, and maintained pathogen collections.
2. Improved knowledge of the molecular basis of pathogenicity and evolution of major agricultural pathogens.
3. Increased knowledge of pathogen biology and life cycles in association with their plant host.

4. Novel disease control strategies that can be developed based on specific molecular targets identified in these studies.
5. New technologies for evaluating the role of pathogen and host genes in pathogenicity and for functional genomics of pathogens will be developed.

PROBLEM STATEMENT 2A: SELECTED ACCOMPLISHMENTS

Output 1: ***Description of pathogen genomes, development of pathogen libraries, and maintained pathogen collections.***

GENOME SEQUENCES COMPLETED:

Viral Pathogens:

Several plant viruses that cause agriculturally important diseases were sequenced by ARS scientists, providing valuable information needed for improved diagnostics, determining evolutionary relationships to other viruses, and providing clues regarding the biological aspects of viruses, including vector associations and host ranges. Selected accomplishments follow:

Sequence of Tobacco rattle virus. Potato is the fourth most important agricultural crop in Michigan, and *Tobacco rattle virus* (TRV), the cause of corky ringspot disease, was recently found in Michigan-grown potatoes. ARS scientists in Prosser, Washington, and Beltsville, Maryland, determined the complete genomic sequence of the Michigan isolate (TRV MI-1) and showed that the North American TRV isolates are different from European isolates. TRV MI-1 is the second North American isolate to be sequenced, and ARS sequence analyses has shown it to be distinct from the previously sequenced North American isolate from Oregon (TRV ORY). This knowledge of the differences between the viruses will enable the development of diagnostic approaches that can distinguish among isolates and are key to devising control measures to reduce crop losses.

Crosslin, J., Hamm, P.B., Kirk, W.W., Hammond, R. 2010. Complete genomic sequence of a tobacco rattle virus isolate from Michigan-grown potatoes. Archives of Virology 155:621-625.

Complete genome sequence of Celery mosaic virus. Celery and carrots, in the family Apiaceae, are important vegetable crops, but many viruses, including *Celery mosaic virus* infect these crops and cause production problems. ARS scientists from Salinas, California, and Beltsville, Maryland, completed the full sequence of this virus, and showed that the virus is most closely related to *Apium virus Y*, *Carrot virus Y*, and *Panax virus Y* in the Potyviridae. These four viruses formed a distinct genetic cluster, indicating they may share a common ancestor in their evolution. The study provides information necessary to assign phylogenetic relationships to this virus. It also provides genetic information for accurate diagnostic and management tools for Potyviruses infecting the Apiaceae.

Xu, D., Liu, H., Li, F., Li, R. 2011. Complete genome sequence of Celery mosaic virus and its relationship to other members of the genus Potyvirus. Archives of Virology 156:917-920.

The complete nucleotide sequence and genome organization of Tomato chlorosis virus. *Tomato chlorosis virus* is one of two important Criniviruses infecting tomato and other hosts. ARS scientists in Salinas, California, completed and annotated the nucleotide sequence of the virus and compared it with those of other members of the genus Crinivirus. Similarities between *Tomato chlorosis virus* and other Criniviruses varies throughout the genome, but the scientists determined that it is more closely related to *Lettuce infectious yellows virus* than to any other Crinivirus. This work establishes a third taxonomic group within the Family Closteroviridae, and provides information needed to distinguish these viruses, which have common hosts and vectors.

Wintermantel, W.M., Hladky, L.L., Gulati Sakhuja, A.N., Li, R., Liu, H., Tzanetakis, I.E. 2009. The complete nucleotide sequence and genome organization of *Tomato infectious chlorosis virus*: A distinct Crinivirus most closely related to lettuce infectious yellows virus. Phytopathology 99: S142.

***Squash vein yellowing virus* genome.** Watermelon vine decline caused by *Squash vein yellowing virus* has been responsible for significant and often complete crop losses in Florida over the past six growing seasons. The complete viral genome was sequenced and analyzed by ARS scientists in Fort Pierce, Florida, who also identified several common cucurbit weeds that can serve as virus reservoirs. The low sequence diversity of this whitefly-transmitted Ipomovirus member of the Potyviridae surveyed in Florida suggests a recent introduction to the United States. Although analysis of the coat protein and two serine proteases (P1a and P1b) sequences for 41 isolates showed little diversity across 7 years of sampling, the analyses revealed two distinct groups of *Squash vein yellowing virus* isolates with low intra-group diversity. Sequence analyses also suggested that recombination had occurred between the virus isolates, similar to other Ipomoviruses, and that the current U.S. population of *Squash vein yellowing virus* has undergone a recent genetic bottleneck and was introduced from elsewhere. This helps explain how vine decline emerged so rapidly and had such a devastating impact on crop losses in a short time.

Webster, C.G., Adkins, S. 2011. Low genetic diversity of Squash vein yellowing virus in wild and cultivated cucurbits in the U.S. suggests a recent introduction. Virus Res. doi:10.1016/j.virusres.2011.11.017 (In Press)

Bacterial Pathogens:

Certain plant pathogenic bacteria are either very difficult to culture, or to date have proven to be recalcitrant to culturing. Sequences of such fastidious microorganisms are helpful in a number of ways, one being the potential to identify the needs for establishment in culture, thus advancing the capacity to test Koch's postulates. Information gained can also help to identify genes involved in pathogenicity, vector

specificity, and other traits that may help to interrupt the infection cycle and thus identify potential targets for unique treatment strategies.

Complete genome sequence of the Huanglongbing bacterium, 'Candidatus Liberibacter asiaticus'. The full genome sequence of '*Candidatus* Liberibacter asiaticus', the causal agent of Huanglongbing, was completed by ARS scientists in Fort Pierce, Florida. Annotation of the 1.23Mb genome revealed 1,186 open reading frames, of which 81 percent had functional assignment. All sequences were submitted to GenBank and shared with other ARS and university researchers. The sequencing of the full genome (www.citrusgreening.org/HLB-GBrowse.html) is facilitating Huanglongbing research and development of new strategies for disease control of this devastating pathogen and related pathogens. The sequence is now being used for improved diagnostics and in efforts to culture the associated organism.

Duan, Y., Zhou, L, Hall, D.G., Li, W., Doddapaneni, H., Lin, H., Liu, L., Vahling, C.M., Gabriel, D.W., Williams, K.P., Dickerman, A., Sun, Y., Gottwald, T. Complete genome sequence of citrus Huanglongbing bacterium, '*Candidatus* Liberibacter asiaticus' obtained through metagenomics. Mol Plant-Microbe Interactions 22:1011–1020.

Complete genome sequence of bacterium associated with zebra chip disease of potato. ARS scientists in Parlier, California, completed the full genomic sequence of '*Candidatus* Liberibacter solanacearum', the causal agent of zebra chip disease of potato, and made the results publically available. Research on zebra chip disease has been hampered by a lack of robust culture methods and paucity of genome sequence information for '*Ca.* Liberibacter solanacearum'. Taxonomically, '*Ca.* Liberibacter solanacearum' is related to the suspected causative agent of citrus Huanglongbing, '*Ca.* Liberibacter asiaticus', yet many genome rearrangements and several gene gains/losses are evident when comparing the two Liberibacter. Sequence analysis revealed a number of unique transporters and pathways, all potentially contributing to zebra chip pathogenesis, providing potential targets for disease management. (www.ncbi.nlm.nih.gov/nuccore/NC_014774?).

Lin, H., Lou, B., Glynn, J.M., Doddapaneni, H., Civerolo, E.L., Chen, C., Duan, Y., Zhou, L., Vahling, C.M. 2011. The complete genome sequence of 'Candidatus Liberibacter solanacearum', the bacterium associated with potato Zebra Chip disease. PLoS One. 6(4):e19135.

Fungal and Oomycetes Pathogens:

As with viral and bacterial plant pathogens, genomic information of fungal pathogens is also valuable. The information can lead to strategies toward interrupting communication between the host and pathogen, and establish new diagnostic methods and control strategies. Cell wall degrading enzymes are often used by fungal pathogens to enter the host, thus providing a potential target for disruption of the pathogenic interactions with the host. Knowledge of genetic relationships can provide clues, through annotation and

comparisons with known relatives, of protein functions that are important in pathogenicity and in basic culturing and manipulation of the pathogen.

Complete genome sequence of Phytophthora infestans. *Phytophthora infestans* is the most destructive pathogen of potato and a model organism for the oomycetes, a distinct lineage of fungus-like eukaryotes related to brown algae and diatoms. ARS scientists in Beltsville, Maryland, and Corvallis, Oregon, contributed to the sequence and annotation of the approximately 240 Mb *P. infestans* genome (http://hdl.handle.net/10113/38731). As the largest and most complex in the chromalveolate lineage, the size of the *P. infestans* genome results from a proliferation of repetitive DNA that accounts for about 74 percent of the genome. Comparison to two other Phytophthora genomes revealed rapid turnover and significant expansions in *P. infestans* genes encoding particular families of secreted proteins, many of which are induced in planta, such as host translocated effectors, for which functions are demonstrated inside plant cells. Functional analysis identified biogenesis effector genes and genes for silencing RNA, which can be used to target the pathogenic response. Genes coding for several cell wall degrading enzymes were also identified that the pathogen uses to gain ingress into the plant as part of the initial infection process. These enzymes will also be important to the biofuels industry, where new cell-wall degrading enzymes are sought.

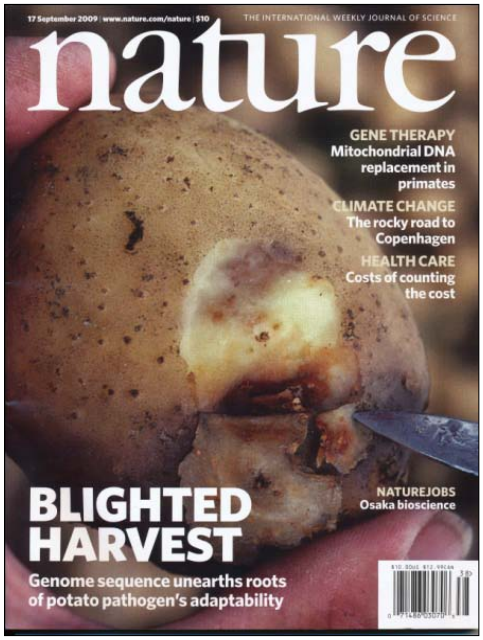

Figure 3: The September 2009 publication in Nature of the *Phytophthora infestans* genome was an historic contribution to understanding one of the world's most destructive pathogens.

Haas, B. J., Kamoun, S., Zody, M. C., Jiang, R. H. Y., Handsake, R. E., Cano, L. M., Grabherr,M., Kodira, C. D., Raffaele, S., Torto-Alalibo, T., Bozkurt, T. O., Ah-Fong, A. M. V., Alvarado, L., Anderson, V. L., Armstrong, M. R., Avrova, A., Baxter, L., Beynon, J., Boevink, P. C., Bollmann, S. R., Bos, J. I. B., Bulone, V., Cai, G., Cakir, C., Carrington, J. C., Chawner, M., Conti, L., Costanzo, S., Ewan, R., Fahlgren, N., Fischbach, M. A., Fugelstad, J., Gilroy, E. M., Gnerre, S., Green, P. J., Grenville-Briggs, L. J., Griffith, J., Grünwald, N. J., Horn, K., Horner, N. R., Hu, C.-H., Huitema, E., Jeong, D.-H., Jones, A. M. E., Jones, J. D. J., Jones, R. W., Karlsson, E. K., Kunjeti, S. G., Lamour, K., Liu, Z., Ma, L., MacLean, D., Chibucos, M. C., McDonald, H., McWalters, J., Meijer, H. J. G., Morgan, W., Morris, P. F., Munro, C. A., O'Neill, K., Ospina-Giraldo, M., Pinzón, A., Pritchard, L., Ramsahoye, B., Ren, Q., Restrepo, S., Roy, S., Sadanandom, A., Savidor, A., Schornack, A., Schwartz, D. C., Schumann, U. D., Schwessinger, B., Seyer, L., Sharpe, T., Silvar, C., Song, J., Studholme, D. J., Sykes, S., Thines, M., van de Vondervoort,

P. J. I., Phuntumart, V., Wawra, S., Weide, R., Win, J., Young, C., Zhou, S., Fry, W., Meyers, B. C., van West, P., Ristaino, J., Govers, F., Birch, P. R. J., Whisson, S. C., Judelson, H. S., and Nusbaum, C. 2009. Genome sequence and analysis of the Irish potato famine pathogen Phytophthora infestans. Nature 461: 393-398.

Sequencing rust fungi. ARS scientists in St. Paul, Minnesota, in a collaborative effort with the Broad Institute of Cambridge, Massachusetts, sequenced the genomes *Melampsora larici-populina* (leaf rust pathogen of poplar), *Puccinia graminis* (the cause of wheat stem rust), *P. triticina* (leaf rust), and *P. striiformis* (stripe rust). Genomic features specific to the obligate biotrophic life-style were identified. This represents the first genome-wide characterization of any rust fungi and provides critical information that will facilitate a better understanding of the complex interactions between these pathogens and their hosts. (www.broadinstitute.org/annotation/genome/puccinia_group/MultiHome.html).

Duplessis, S., Cuomo, C.A., Lin, Y., Aerts, A., Tisserant, E., Veneault-Fourrey, C., Joly, D., Hacquard, S., Amselem, J., Cantarel, B., Chiu, R., Couthinho, P., Feau, N., Field, M., Frey, P., Gelhaye, E., Goldberg, J., Grabherr, M., Kodira, C., Kohler, A., Kues, U., Lindquist, E., Lucas, S., Mauceli, E., Morin, E., Murat, C., Pearson, M., Quesneville, H., Rouhier, N., Sakthikumar, S., Schmutz, J., Selles, B., Shapiro, H., Tangay, P., Tuskan, G.A., Van De Peer, Y., Henrissat, B., Rouze, P., Schein, J., Dodds, P.N., Zhong, S., Hamelin, R.C., Birren, B.W., Grigoriev, I.V., Szabo, L.J., Martin. 2011. Obligate biotrophy features unraveled by the genomic analysis of the rust fungi, *Melampsora larici-populina* and *Puccinia graminis* f. sp. *tritici*. Phytopathology.108:9166-9171.

Complete genome sequence for Mycosphaerella graminicola. ARS scientists in Lafayette, Indiana, with their team of collaborators, completed the sequence genome for *Mycosphaerell graminicola*, the fungal cause of *Septoria tritici* blotch and a global threat to wheat production. Control of the disease has been hampered by a limited understanding of the genetic and biochemical bases of pathogenicity, including mechanisms of infection and of resistance in the host. A group of eight chromosomes were shown to be dispensable, both experimentally and through sequence annotation. The latter, called collectively the "dispensome," were dynamic in field and progeny isolates and may represent an evolutionary response to evade detection by plant defense mechanisms. They were distinct in structure, gene, and repeat content, but contained parts from each core chromosome, suggesting ancient horizontal gene transfer. The genome of *M. graminicola* had far fewer genes for cell wall-degrading enzymes and secondary metabolites compared to other plant pathogens, and thus are considered more similar to endophytes. The "stealth pathogenesis" of *M. graminicola* – shown by a long latency period while it invades the host – probably involves degradation of proteins rather than carbohydrates to evade host defenses during the biotrophic stage of infection and may have evolved from an endophytic ancestor. The finished genome of *M. graminicola* provides a gold standard for this class of fungi, the largest and most ecologically diverse group of Ascomycetes with approximately 20,000 species, classified in 11 orders and 90 families. This work

provides a huge advantage for comparative genomics for identifying the genetic basis of highly divergent lifestyles among pathogenic fungi.

Goodwin, S. B., M'Barek, S. B., Dhillon, B., Wittenberg, A. H. J., Crane, C. F., Hane, J. K., Foster, A. J., Van der Lee, T. A. J., Grimwood, J., Aerts, A., Antoniw, J., Bailey, A., Bluhm, B., Bowler, J., Bristow, J., van der Burgt, A., Canto-Canché, B., Churchill, A. C. L., Conde-Ferràez, L., Cools, H. J., Coutinho, P. M., Csukai, M., Dehal, P., De Wit, P., Donzelli, B., van de Geest, H. C., van Ham, R. C. H. J., Hammond-Kosack, K. E., Henrissat, B., Kilian, A., Kobayashi, A. K., Koopmann, E., Kourmpetis, Y., Kuzniar, A., Lindquist, E., Lombard, V., Maliepaard, C., Martins, N., Mehrabi, R., Nap, J. P. H., Ponomarenko, A., Rudd, J. J., Salamov, A., Schmutz, J., Schouten, H. J., Shapiro, H., Stergiopoulos, I., Torriani, S. F. F., Tu, H., de Vries, R. P., Waalwijk, C., Ware, S. B., Wiebenga, A., Zwiers, L.-H., Oliver, R. P., Grigoriev, I. V., and Kema, G. H. J., 2011. Finished genome of the fungal wheat pathogen *Mycosphaerella graminicola* reveals dispensome structure, chromosome plasticity and stealth pathogenesis. PLoS Genet 7(6): e1002070.

Output 2: ***Improved knowledge of the molecular basis of pathogenicity and evolution of major agricultural pathogens.***

A new, naturally occurring hybrid virus of sweetpotato is more severe than known viruses. In sweetpotato field trials where germplasm is routinely screened for resistance to economically limiting viruses, a new member of the whitefly-transmitted Begomovirus group was detected that is more severe than common sweetpotato viruses. Viruses in the Begomovirus group sometimes produce natural hybrids in the field, and this was shown to be a case of two begomoviruses – *Sweet potato leaf curl virus* and *Sweet potato leaf curl Georgia virus*. In collaboration with Alcorn State University, ARS scientists in Charleston, South Carolina, discovered that these viruses hybridized to form a new, more destructive virus for which resistance is not currently available. This new virus can result in a 20-80 percent crop yield reduction in current U.S. sweetpotato cultivars. A broad spectrum diagnostic test was developed by ARS scientists that will now detect all members of the sweetpotato Begomovirus group enabling screening of sweetpotato germplasm for new sources of disease resistance.

Zhang, S., Ling, K. 2011. Genetic diversity of sweetpotato Begomoviruses in the United States and identification of a natural recombinant between Sweet potato leaf curl virus and Sweet potato leaf curl Georgia virus. Archives of Virology.156: 955-968

Characterization of the mating type locus in Cercospora beticola. Cercospora leaf spot, caused by the hemibiotrophic fungal pathogen *Cercospora beticola*, is the most economically damaging foliar disease of sugarbeet worldwide, costing sugarbeet producers millions of dollars annually. Although most *C. beticola* populations display characteristics reminiscent of sexual recombination, no teleomorph has been described. ARS scientists in Fargo, North Dakota, characterized the mating type genes of *C. beticola*, genes that are known to be required for sexual reproduction. Their results suggest that *C. beticola* mating type genes are still active and may play a role in sexual reproduction of this fungus. Plant breeders, when selecting parents for

developing disease resistant sugarbeet, and plant pathologists can apply this knowledge to help explain how *C. beticola* is able to develop fungicide resistance.

Bolton, M. 2011. Distribution and characterization of mating type genes in field populations of *Cercospora beticola* from USA. Fungal Biology In press.

Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. Fusarium species are among the most economically important and toxigenic group of plant pathogenic fungi. To understand the genetic basis for pathogenicity in the genus Fusarium, ARS scientists in St. Paul, Minnesota, compared the genomes of three phenotypically diverse species: *F. graminearum, F. verticillioides,* and *F. oxysporum* f. sp. *lycopersici.* Genomic analysis revealed lineage-specific genomic regions (accounting for over one quarter of the genome) that are rich in transposons and genes with distinct evolutionary profiles, but related to pathogenicity, indicative of horizontal acquisition. Experimentally, the scientists demonstrated the transfer of two lineage-specific chromosomes between strains of *F. oxysporum,* converting a non-pathogenic strain into a pathogen. The results demonstrate that, although genome sequences are highly similar in the three species, important differences exist, including some required for production of different toxic metabolites, whereas others are important for ability to cause disease on crop hosts. These findings provide information that plant breeders and plant biotechnologists can use to improve resistance of crops to diseases caused by Fusarium and reduce mycotoxin contamination of crops.

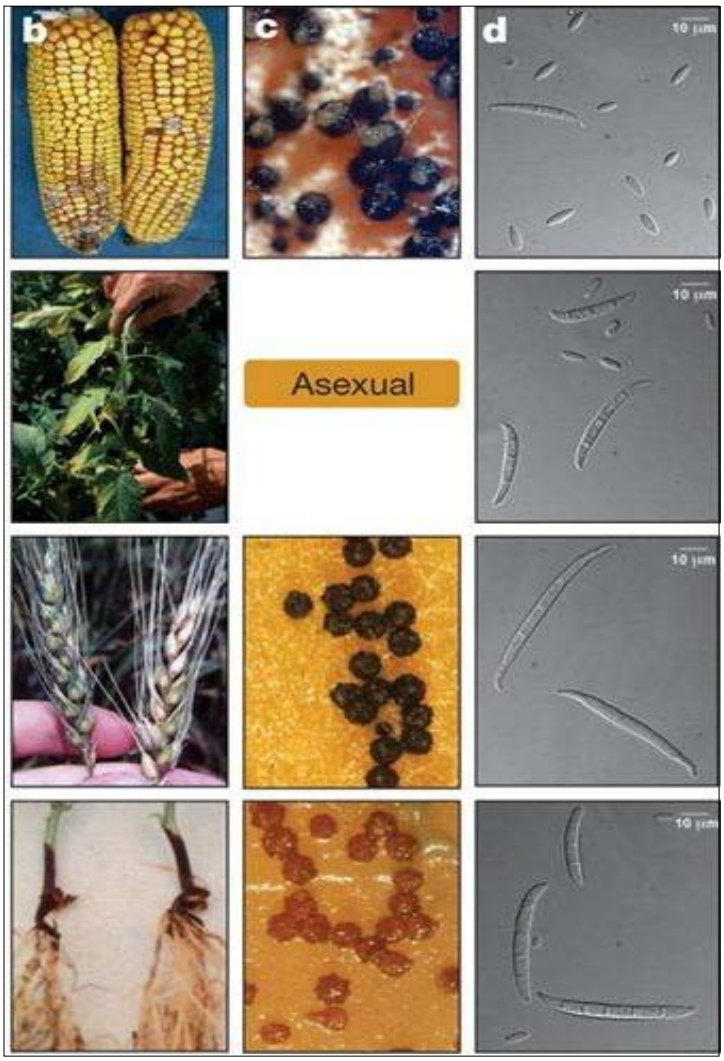

Figure 4: Three Fusarium species differ in biological properties such as host range (**b**), perithecial stages (**c**) and microconidia morphology (**d**). Phenotypic variation is shown within the genus *Fusarium* on different hosts: **b:** disease symptoms of (top to bottom) kernel rot of maize (*F. verticillioides; Fv*); wilt of tomato (*F. oxysporum f. sp. lycopersici;Fol*); head blight of wheat (*F. graminearum; Fg*); and root rot of pea (*F. solani*; *Fs*). **c:** the perithecial states of *Fv* (*Gibberella moniliformis*), *Fol* (no sexual state), *Fg* (*G. zeae*), and *Fs* (*Nectria haematococca*). **d:** micro- and macroconidia of *Fv*, *Fol*, *Fg,* and *Fs. S*cale bars = 10 µm. *Fg* produces only macroconidia.

Ma, L., Borkovich, K.A., Coleman, J.J., Daboussi, M., Pietro, A.D., Dufresne, M., Freitag, M., Grabherr, M., Henrissat, B.,

Kang, S., Park, J., Rep, M., Shim, W., Woloshuk, C., Xie, X., Xu, J., Antoniw, J., Baker, S.E., Bluhm, B.H., Breakspear, A., Brown, D.W., Butchko, R.A., Chapman, S., Coulson, R., Coutinho, P.M., Danchin, E., Diener, A., Gale, L., Gardiner, D., Goff, S., Hammond-Kosack, K.E., Hilburn, K., Hua-Van, A., Jonkers, W., Kazan, K., Kodira, C.D., Koehrsen, M., Kumar, L., Lee, Y., Li, L., Manners, J., Miranda-Saavedra, D., Mukherjee, M., Park, G., Park, J., Park, S., Proctor, R., Regev, A., Ruiz-Roldan, M., Sain, D., Sakthikumar, S., Sykes, S., Schwartz, D.C., Turgeon, G., Wapinski, I., Yoder, O., Young, S., Zeng, Q., Zhou, S., Galagan, J., Cuomo, C.A., Kistler, H.C. 2010. Comparative Genomics Reveals Mobile Pathogenicity Chromosomes in Fusarium. Nature 464:367-373.

RNA silencing in Potato spindle tuber viroid. Diseases caused by viroids are characterized by marked changes in host gene expression and activation of an RNA-based defense system known as RNA silencing which plays an important role in symptom development during viroid infection. Just how and where the small viroid-related RNAs are generated during the infection process is not fully understood. To better understand the relationship between RNA silencing and the appearance of visible disease symptoms, ARS scientists in Beltsville, Maryland, determined the presence of three size classes of viroid-specific small RNAs in plants infected with *Potato spindle tuber viroid*. Sequence analysis demonstrated the presence of a previously undescribed cluster of small RNAs derived primarily from (-) strand *Potato spindle tuber viroid* RNA from a portion of the pathogenicity and central domains. The process by which viroid-specific small RNAs are generated appears to be more complicated than previously believed, possibly involving multiple Dicer-like activities, viroid RNA substrates, and subcellular compartments. Silencing of viroid-caused diseases may provide new means of disease management for this group of pathogens.

Machida, S., Yamahata, N., Watanuki, H., Owens, R.A., Sano, T. 2007. Successive accumulation of two size classes of viroid-specific small RNA in potato spindle tuber viroid-infected tomato plants. Journal of General Virology 88:3452-3457.

Golden nematode mimics plant signaling peptides required for infection. The golden nematode (a species of the potato cyst nematode) is a devastating quarantine pest that threatens the U.S. potato industry. To infect potato roots, the nematode secrets proteins into root cells to transform them into a specialized site for feeding. Establishment of the specialized feeding site is essential for the growth and development of the nematode. Identification of nematode-secreted proteins and understanding of their functions is necessary for understanding the molecular basis of feeding site formation, and for developing novel nematode control strategies. The CLE protein family, once thought to be plant-specific, was identified and cloned from plant-parasitic cyst nematodes by ARS scientists in Ithaca, New York. Plant CLE proteins are a family of signaling peptide ligands that play diverse roles in plant growth, development, and signaling between cells. The discovery of CLEs from the golden nematode and other plant-parasitic nematode species suggests that nematodes secret plant peptide mimics to manipulate plant developmental pathways for their own parasitic advantage. Plant receptor proteins were also discovered that recognize nematode-secreted CLE proteins, and this recognition is critical for successful feeding site formation within host roots. The discovery of nematode CLEs represents an extraordinary example of cross-kingdom adaptation between plants and nematodes. The knowledge developed will provide host targets useful for generating novel forms of nematode resistance in crop plants.

Guo, Y., Ni, J., Denver, R., Wang, X., and Clark, S.E. 2011. Mechanisms of molecular mimicry of plant CLE peptide ligands by the parasitic nematode *Globodera rostochiensis*. Plant Physiology 157: 476–484.

Understanding genetic variability of Fusarium yellows in sugar beet. Fusarium yellows disease, caused by the soil-borne fungus *Fusarium oxysporum f. sp. betae* (Fob), can lead to significant yield losses in sugar beet, and is one of the major targets in breeding programs for resistance development. Although genetic resistance provides some control, growers have reported crop failures when resistant varieties are grown in different parts of the country, potentially due to the variability of local Fob populations. ARS scientists in East Lansing, Michigan, and Fort Collins, Colorado, analyzed 86 Fusarium isolates from different geographic areas, including nonpathogenic and pathogenic species from sugar beet, dry bean, and spinach, and Fusarium DNA from Europe. Sequence data from selected genes were used to examine whether Fusarium diversity is related to geographic origin and/or pathogenicity. Analyses revealed no clades based on geographic origin and a single clade consisting exclusively of pathogens. Based on these findings, the Fob population is highly polyphyletic and most likely cannot be classified into distinct races, suggesting that the resistance breeding efforts for sugarbeet should focus on pyramiding resistance genes for durability.

Hill, A.L., Reeves, P.A., Larson, R.L., Fenwick, A.L, Hanson., L.E. and Panella, L. 2010. Genetic variability among isolates of *Fusarium oxysporum* from sugar beet. Plant Pathology Doi: 10.1111/j.1365-3059.2010.02394.x

Output 3: ***Increased knowledge of pathogen biology and life cycles in association with their plant host.***

Replication of the aster yellows phytoplasma within its insect vector. Aster yellows is an economically important pathogen of carrot, and its phytoplasma is transmitted by the aster leafhopper, *Macrosteles quadrilineatus*. ARS scientists in Madison, Wisconsin, in collaboration with researchers at the University of Wisconsin-Madison, developed a quantitative real-time reverse transcription polymerase chain reaction PCR (qPCR) assay to measure aster yellows concentration in insect deoxyribonucleic acid (DNA) extracts. The research demonstrated that the aster yellows phytoplasma efficiently replicates within the leafhopper reaching a maximum titer in 10 days. This new technique will facilitate examination of the biological factors governing aster yellows replication in the leafhopper and will provide information for management strategies of this disease in the field.

Frost, K.E., Willis, D.K., German, T.L. 2010. Variation in aster yellows phytoplasma (*Candidatus Phytoplasma Asteris*) titer in its insect vector, *Macrosteles Quadrilineatus* [abstract]. Entomological Society of America Proceedings 519.

Mixed infections of mosaic viruses increase infection levels in susceptible wheats. The hard red winter wheat 'Mace' is an ARS cultivar with resistance to *Wheat streak mosaic virus* (WSMV). WSMV and the newly emergent *Triticum mosaic virus* are transmitted by the same wheat curl mite vector, so that mixed infections by the two viruses are now anticipated to be common, and an increase in virulence is possible. ARS scientists in Lincoln, Nebraska, showed that there was increased virus content in WSMV-susceptible varieties when they were infected

by both viruses compared to each one individually, which could enhance their natural spread by mites. Consequently, growers should plant WSMV-resistant varieties where available, and follow current cultural disease control practices such as delayed planting of susceptible varieties in the fall. This research has encouraged other wheat breeders to incorporate the virus resistance gene from Mace into their own breeding programs for WSMV and *Triticum mosaic virus*. Commercial sales of Mace seed occurred in time for fall 2011 planting, and wheat producers in Texas, Oklahoma, and Nebraska will harvest their first crop in 2012.

Graybosch, R. A., Peterson, C. J., Baenziger, P. S., Baltensperger, D. D., Nelson, L. A., Jin, Y., Kolmer, J. A., Seabourn, B. W., French, R., Hein, G. L., Martin, T.J., Beecher, B., Schwarzacher T,, and Heslop-Harrison, P. 2009. Registration of 'Mace' hard red winter wheat. Journal of Plant Registrations 3:51-56.

Tatineni, S., Graybosch, R. A., Hein, G. L., Wegulo, S.N., French, R. 2010. Wheat cultivar-specific disease synergism and alteration of virus accumulation during co- infection with wheat streak mosaic virus and triticum mosaic virus. Phytopathology 100:230-238.

Immunity of cotton bolls to stink bug-vectored bacterial infections. A bacterial seed and boll disease has recently emerged as an important obstacle to efficient cotton production in the southeastern United States. Seed and boll rot disease causes significant yield losses ranging from 10 to 15 percent. ARS scientists in College Station, Texas, found that although feeding behavior by the southern green stink bug facilitates development of the disease in young cotton bolls, the bolls become essentially immune as early as 3 weeks into boll development. This finding documents the timeline of cotton boll susceptibility/resistance and will facilitate efforts to manage the disease, through timely insect control strategies, to assure effectiveness of disease control while avoiding unnecessary and expensive control measures.

Esquivel, J.F., Medrano, E.G., Bell, A.A. 2011. Southern green stink bugs (Hemiptera: Pentatomidae) as vectors of pathogens affecting cotton bolls – A brief review. Southwest Entomol. 35:457-461.

Medrano, E.G., Esquivel, J.F., Bell, A.A., Greene, J., Roberts, P., Bachelor, J., Marois, J.J., Wright, D.L., Nichols, R.L., Lopez, J. 2009. Potential for Nezara viridula (Hemiptera: Pentatomidae) to transmit bacterial and fungal pathogens into cotton bolls. Curr. Microbiol. 59:405-412.

Output 4: ***Novel disease control strategies that can be developed based on specific molecular targets identified in these studies.***

Fungal virulence factors identified in the blue mold-pome fruit pathosystem. Postharvest diseases, such as blue mold of apples and pears, cause significant losses to fresh market production. Growers and packers sometimes allow temperatures to rise to reduce cooling costs, increasing the risk of decay by blue mold. ARS scientists in Beltsville, Maryland, extracted and purified polygalacturonases (PG) from *Penicillium expansum* and *P. solitum* found on decayed pear and apple fruit tissue. Both fungi produced a single PG in decayed tissue that differs in the ability to function at various pHs and incubation temperatures. This research reinforced the need to maintain proper storage temperature for apples and pears. This fundamental research should lead to identifying PG inhibitors toward specific decay control strategies that can be applied directly to the fruit surface as a coating to reduce fungicide residues on fruit.

Jurick II, W.M., Vico, I., Gaskins, V.L., Garrett, W.M., Whitaker, B.D., Janisiewicz, W.J., and Conway, W.S. 2010. Purification and biochemical characterization of polygalacturonase produced by *Penicillium expansum* during postharvest decay of 'Anjou' pear. Phytopathology 100:42-48.

Vico, I., Jurick II, W.M., Camp, M.J., Janisiewicz, W.J., and Conway, W.S. 2010. Temperature suppresses decay on apple fruit by affecting *Penicillium solitum* conidial germination, mycelial growth and polygalacturonase activity. Plant Pathology Journal.9129-133.

Output 5: ***New technologies for evaluating the role of pathogen and host genes in pathogenicity and for functional genomics of pathogens.***

Plant viral-based vectors and gene constructs for plant disease control. Plant virus-based transient expression vectors provide molecular tools to facilitate the study of viroid- and virus-host interactions, pathogen movement and disease development, and novel disease control strategies and products for plant and animal diseases. *Cucumber mosaic virus* has provided a valuable tool, due to its wide host range, for expression of foreign genes in plants, without having to genetically modify the host. A novel *Cucumber mosaic virus*-based vector system was constructed for transient expression of foreign genes in a wide number of plant hosts that allow rapid screening of potential therapeutic compounds for bacterial diseases. ARS scientists in Beltsville, Maryland, demonstrated the antimicrobial activities of plant defense proteins (snakin-1 and defensin) by transient expression in plant virus-based vectors against bacteria that cause potato ring rot and anthracnose, and showed in vivo coproduction of these hybrid proteins is a promising strategy for plant defense applications. Development of recombinant virus-based DNA molecules for expression of foreign genes in plants makes possible the rapid testing of antimicrobial proteins in vegetable crops, and will allow future deployment of new disease control strategies against a wide range of pathogens

Kovalskaya, N., Zhao, Y., Hammond, R. 2011. Antibacterial and antifungal activity of a snakin-defensin hybrid protein expressed in tobacco and potato plants. The Open Plant Science Journal 5:29-42.

Salyaev, R.K., Rekoslavskaya, N.I., Stolbikov, A.S., Hammond, R., Shchelkunov, S.N. 2009. Retention of the ability to synthesize HIV-1 and HBV antigens in generations of tomato plants transgenic for the TBI-HBS gene. Doklady Biochemistry and Biophysics 425:120-123.

A new platform for antigen display in plants. Agricultural losses due to plant and animal diseases necessitate the development of novel reagents for detection and control of the pathogens that cause the disease. Plant viruses provide useful templates for this purpose, and can be applied to both plant and animal disease control. Novel nano-material tools were developed by ARS scientists at Beltsville, Maryland, using plant virus-like particles as protein cages for uses in biotechnology, genetically engineered from *Maize rayado fino virus*. To produce a platform for these reagents, the scientists demonstrated that these chemically-modified plant virus-like particles can serve as platforms for the display of diverse molecules such as antigenic peptides and fluorescent dyes. Similar expression systems developed by ARS scientists have been used to develop vaccines for animals that are produced in tomato plants, including Newcastle Disease

virus vaccine; a functional bovine receptor protein as a therapeutic agent for coliform mastitis; vaccine antigens for the major Hepatitis B surface antigen; HIV-1 antigens; and sheep pox virus

Natilla, A., Hammond, R.W. 2011. *Maize rayado fino virus* virus-like particles expressed in tobacco plants: A new platform for cysteine selective bioconjugation peptide display. J Virol Methods. 178:209-15.

PROBLEM STATEMENT 2B: ***Plant-Microbe-Vector Interactions***

Many plant viruses, mollicutes, and fastidious bacteria are disseminated by insect vectors, but some are also vectored by parasitic plants, mites, fungi, or nematodes. The molecular and cellular mechanisms of transmission are poorly understood and include complex interactions between the pathogen, vector, and host plant.

ARS scientists have demonstrated leadership and made crucial contributions towards a better understanding of the pathogen-host interaction of several economically limiting diseases – Huanglongbing, zebra chip, Pierce's disease, and Sudden Oak Death caused by *Phytophthora ramorum.* Knowledge of disease etiology and plant-pathogen-vector interactions provides opportunities to disrupt the disease cycle and provide potential management opportunities. These research advances are diverse in scope and include both basic and applied approaches.

The NP 303 Action Plan identified one anticipated Output that was expected to result from research addressing the needs expressed in Problem Statement 2B. Accomplishments that illustrate the product for this problem statement follow.

ANTICIPATED OUTPUT IN ACTION PLAN:

1. New technologies for pathogen and vector management and control.

PROBLEM STATEMENT 2B: SELECTED ACCOMPLISHMENTS

Output 1: ***New technologies for pathogen and vector management and control.***

National coordination of Huanglongbing disease control. Huanglongbing, transmitted by the Asian citrus psyllid, is the most serious threat to the U.S. citrus industry, since it was first detected in 2005. The disease has already greatly affected the Florida citrus industry, causing millions of dollars in damage and lost revenue. Currently, there is no commercial source of host resistance to this disease. The Asian citrus psyllid vector of the bacterium ('*Candidatus* Liberibacter asiaticus') associated with Huanglongbing is found in Texas and California, but the disease has not become established there yet. In response to requests from U.S. citrus industry representatives, ARS, APHIS, and NIFA worked with industry partners to built a framework for a coordinated interdisciplinary research response to Huanglongbing that includes cultural management; psyllid suppression; early detection and new diagnostic tools; removal of infected trees; disease resistance development in commercial citrus cultivars and rootstocks; and potential use of therapeutics to maintain healthy citrus trees. With the unified strategic plan in place, ARS and its partners are targeting research that will have the greatest impact on meeting goals for a

productive, sustained citrus industry. Under the Citrus Health Response Program established by APHIS, this national group established an annual Citrus Health Research Symposium, and held its second meeting in October 2011.

ARS scientists and their collaborators made significant contributions toward the full genomic sequences of '*Ca.* Liberibacter asiaticus', the Asian citrus psyllid, and that of *Poncirus trifoliata* (an important citrus rootstock, also known as Carizzo). All three sequences are available publicly (see Web links below). The bacterial sequence has provided information leading to more sensitive and specific diagnostic tools for use in the citrus host and the vector that have been used extensively by researchers and regulators. The vector sequence is being used to develop small molecules that interfere with the psyllid life cycle (interfering RNA). The rootstock sequence has already helped to identify citrus genes for use in developing genetically modified citrus using only citrus-specific genes. Genes and control elements derived from the citrus genome are being trialed in the effort to produce "intragenic" sources of resistant cultivars. In addition, improved spray technology has been transferred to growers that significantly reduce the volume and amount of pesticides needed for psyllid control, a significant cost savings for growers.

'*Ca.* Liberibacter asiaticus' – http://citrusgreening.org/HLB-at-NCBI.html
Asian citrus psyllid – www.psyllid.org/node/10
Carizzo rootstock – http://citrus.pw.usda.gov/

Following are selected examples of the diverse accomplishments made by NP 303 ARS scientists in Fort Pierce, Florida; Beltsville, Maryland; and Parlier, Riverside, and Albany, California, in Huanglongbing research:

Seed transmission of 'Ca. Liberibacter asiaticus' in citrus. Seed transmission of the Huanglongbing bacterium has been a controversial subject and has important implications for certified citrus nurseries that depend on seed propagation. '*Ca.* Liberibacter asiaticus' was found only to colonize seed coats and not the embryo. Peeled seed that was germinated and grown out showed no apparent infection of the embryo or of the emergent seedling. A fluorescent in situ hybridization was developed to observe fluorescently-labeled '*Ca.* Liberibacter asiaticus' cells in plant tissues, allowing visual assessment of the bacterium in seed coat tissues only. This work was presented at the Second Citrus Health Research Symposium in 2011.

Genetic relationships of wild citrus relatives. The ARS National Plant Germplasm System maintains a valuable citrus germplasm collection at Riverside, California, and ARS scientists there have been evaluating the diverse citrus germplasm for potential sources of resistance to '*Ca.* Liberibacter asiaticus' for the purpose of breeding commercial varieties resistant to Huanglongbing. In collaboration with researchers with the University of California-Riverside, the ARS scientists analyzed the genetic relationship of 61 species of 35 genera of the Rutaceae related to citrus according to the variability in the nucleotide sequence of a specific gene. The phylogenetic information generated provides a source of single-nucleotide polymorphism (SNP) markers for rapid and accurate assays of crop genetic variability needed to design efficient and effective curatorial programs and novel breeding strategies.

DNA sequence analyses of its prophage genes indicate diverse and variable 'Ca. Liberibacter asiaticus' isolates in Florida. The variations among the prophage from global isolates indicate multiple '*Ca.* Liberibacter asiaticus' populations exist. This is perhaps best explained by multiple source introductions of the bacterium into Florida.

Zhou, L., Powell,, C.C., Hoffman, M.T., Li, W., Fan, G., Liu, B., Lin, H., and Duan, Y. 2011 Diversity and plasticity of the intracellular plant pathogen and insect symbiont "*Candidatus* Liberibacter asiaticus" as revealed by hypervariable prophage genes with intragenic tandem repeats. Appl. Environ. Microbiol. 6663-6673.

Therapeutic treatments for Huanglongbing-infected hosts. Using periwinkle as a test plant inoculated with '*Ca.* Liberibacter asiaticus' by dodder, the combination of penicillin and streptomycin was effective in eliminating or suppressing the pathogen in periwinkle, and provided a therapeutically effective level of control for a much longer period of time than when administering either antibiotic separately. Application of the penicillin and streptomycin to citrus via trunk injection or root soaking also eliminated or suppressed the '*Ca.* Liberibacter asiaticus' in the Huanglongbing-affected citrus plants. This provides a potential therapeutic treatment for the managing Huanglongbing in infected trees that cannot be destroyed.

Zhang, M. Q., Powell, C. A., Zhou, L. J., He, Z. L., Stover, E., and Duan, Y. P. 2011. Chemical compounds effective against the citrus Huanglongbing bacterium '*Candidatus* Liberibacter asiaticus' in planta. Phytopathology 101:1097-1103.

Zebra chip of potato. A disease of potato, zebra chip, vectored by the potato psyllid and with a similar association to the virus '*Ca*. Liberibacter solanacearum', is a significant problem for the potato industry. ARS scientists in Wapato, Washington, demonstrated that the potato psyllid was indeed the vector, and, with their university collaborators, are evaluating potato germplasm for resistance to this disease. Understanding the host-vector relationships is essential in controlling zebra chip in an annual crop like potato. Insecticides for the potato psyllid have been instrumental in managing zebra chip.

Buchman, J.L., Sengoda, V.G., and Munyaneza, J.E. 2011. Vector transmission efficiency of Liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in zebra chip potato disease: Effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104:1486-1495.

Management strategies for Pierce's disease of grape and diseases caused by Xylella fastidiosa. Pierce's disease, caused by the xylem-limited bacterium *Xylella fastidiosa* (Xf), is an economically important disease affecting U.S. grape production and numerous horticultural crops. In California, prevalence and incidence of Pierce's disease increased following introduction and establishment of the highly efficient vector, the glassy-winged sharpshooter, *Homalodisca vitripennis*. Presently, Pierce's disease is managed in California via an area-wide surveillance and insecticide application program aimed at suppressing glassy-winged sharpshooter populations in citrus (the most common feeding/oviposition host) and urban landscape plants. Development of an integrated management program for Pierce's disease requires detailed knowledge of host-pathogen-vector-environment interactions. ARS research on

Pierce's disease is designed to address knowledge gaps in the biology, ecology, and genetics of Xf, sharpshooter vectors, and host plants. Below are selected examples of developments from ARS projects on Pierce's disease:

Deficit irrigation of citrus affects glassy-winged sharpshooter population density. Current management practices for glassy-winged sharpshooter, the efficient vector for Xf in commercial orchards rely on application of insecticides. ARS scientists in Parlier, California, evaluated cultural practices (deficit irrigation and fertilization) in citrus that affect glassy-winged sharpshooter populations. The results indicated that moderate water stress increased sharpshooter population density, whereas severe water stress reduced population density. This suggests that the physiological state of citrus can be manipulated to reduce sharpshooter population density, and points to the effect deficit irrigation has as an additional strategy for suppression of vector population density in an integrated management plan.

Krugner, R., Groves, R. L., Johnson, M. W., Flores, A. P., Hagler, J. R., and Morse, J. G. 2009. Seasonal population dynamics of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) in sweet orange trees maintained under continuous deficit irrigation. Journal of Economic Entomology 102: 960-973.

Nadel, H., Seligmann, R., Johnson, M. W., Hagler, J. R., Stenger, D. C., and Groves, R. L. 2008. Effects of citrus and avocado irrigation and nitrogen-form soil amendment on host selection by adult *Homalodisca vitripennis*. Environmental Entomology 37:787-795.

High throughput detection of Xylella fastidiosa causing almond leaf scorch. *Xylella fastidiosa*, the causal agent of almond leaf scorch disease, is currently re-emerging as a serious disease in California. Pathogen detection is a critical step for disease diagnosis because the disease symptoms are often confused with salt toxicity and other environmental stresses. ARS scientists in Parlier, California, have developed a simple PCR method to detect Xf directly from pulverized freeze-dried tissue that showed a 92.8 percent correlation with the "gold standard." Considering the simplicity, the new, modified PCR method is a valuable option for high throughput rapid detection of Xf in the ALSD epidemiological studies. This procedure was used by extension pathologists at the University of Kentucky and in Europe to evaluate Xf infection in several crops hosts, in comparison to standard methods and to help improve current methods.

Chen, J., Livingston, S., Groves, R.L., Civerolo, E.L. 2008. High throughput PCR detection of *Xylella fastidiosa* directly from almond tissues. Journal of Microbiological Methods 73:57-61.

Development of Wheat streak mosaic virus expressing a fluorescent protein as a genetic tool. The use of viral vectors to transiently express proteins and other products in plants for examining the functions of plant genes by the process called virus-induced gene silencing has been growing rapidly. Transient expression of foreign genes in plants through viral-vectors is considered to be an alternative approach to stable transformation of plants because of the difficulty to transform some plant species, including cereal crops. ARS scientists in Lincoln, Nebraska, have developed an improved virus gene expression vector based on *Wheat streak mosaic virus* (WSMV). This is the only virus vector capable of expressing foreign genes in wheat and other cereals, and

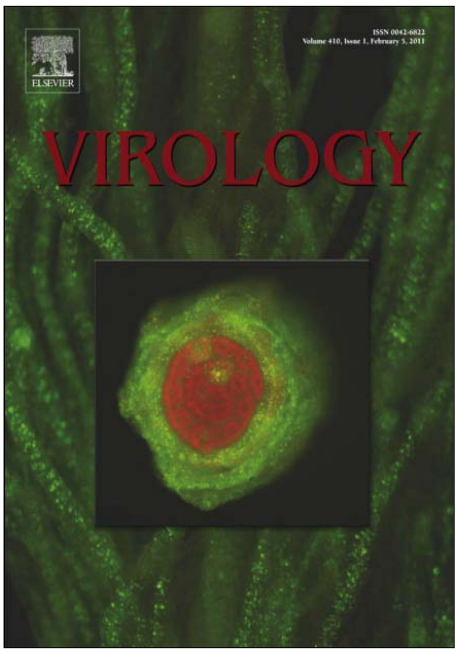

Figure 5: Confocal laser scanning microscope picture showing expression of green fluorescent protein (GFP) in a stem cross section with fluorescence in outer leaf sheaths of wheat infected with *Wheat streak mosaic virus* at 14 days past infection. Note the young leaf located in the center of the stem is mostly free from GFP fluorescence.

provides a new research tool. The numerous applications of this new research tool include the ability to examine the function of diverse cereal gene functions, since WSMV infects a wide range of cereal crops. WSMV-green fluorescent protein vectors were found to be highly stable in wheat for more than 120 days after inoculation, which was close to the life span of wheat. WSMV-green fluorescent protein vectors were also transmitted by the wheat curl mite (the vector of WSMV), infected the same range of cereal hosts, and green fluorescent protein fluorescence was detected in most wheat tissues, similar to the wild-type virus. This provides an easy way of screening germplasm for virus resistance and for examining virus-mite interactions and mechanisms of transmission**.**

Tatineni, S., Mcmechan, A., Hein, G., French, R.C. 2011. Efficient and stable expression of GFP through Wheat streak mosaic virus-based vectors in cereal hosts using a range of cleavage sites. Virology 410: 268-281.

Ecology and management of whitefly-transmitted viruses of vegetable crops in Florida. A variety of fresh market vegetables, including watermelon and tomato, are economically important crops in Florida. Management strategies for the diversity of whitefly-transmitted viruses infecting these crops require multiple approaches. ARS scientists in Fort Pierce, Florida, conducted surveys in the region to identify alternative hosts for four viruses (*Tomato yellow leaf curl virus*, *Cucurbit leaf crumple virus*, *Cucurbit yellow stunting disorder virus* and *Squash vein yellowing virus*), and the effectiveness of insecticides and silver plastic mulch to manage the whitefly vectors and to evaluate potential sources of *Squash vein yellowing virus* resistance on disease management. ARS scientists developed a comprehensive map of 82,928 acres of vegetable fields comprising the majority of the southwest Florida vegetable production area to identify hot spots and reservoir crops for viruses and whiteflies. The viruses are being introduced independently by whiteflies, although the whiteflies may be emigrating from the same source, with secondary spread being dominated by within-field populations of whiteflies. This continuing research will provide critical information necessary to develop decision management strategies.

Adkins, S., Webster, C.G., Kousik, C.S., Webb, S.E., Roberts, P.D., Stansly, P.A., Turechek, W.W. 2011. Ecology and management of whitefly-transmitted viruses of vegetable crops in Florida. Virus Res. 159:110-4.

New tool to identify gene function. Fusarium head blight (FHB) is a major disease affecting production of wheat and barley. Incorporation of genetic resistance is the most effective strategy for combating this disease, and although vigorous searches have found resistance, researchers have failed to identify a specific gene responsible for the FHB resistance. ARS scientists in West Lafayette, Indiana, have developed a virus-induced gene silencing (VIGS) assay that makes

it possible to observe the consequences of switching off genes in normally FHB-resistant plants. The FHB-VIGS assay has proven success in identifying a range of genes contributing to FHB resistance. This information is now being used to understand the mechanisms of FHB resistance and for breeding wheat and barley with improved FHB resistance.

Scofield, S.R., Gillespie, M., Cakir, C. 2010. Rapid determination of gene function by virus-induced gene silencing in wheat and barley. Crop Science 50:77-84.

PROBLEM STATEMENT 2C: ***Population Dynamics, Spread, and Epidemiology of Pathogens.***

Epidemiological studies are essential parts of plant disease detection, etiology, and management strategy development. Models can be developed to predict disease outbreaks and optimize management strategies that are cost effective and minimize yield losses. For new and emerging diseases, epidemiological studies are especially important to determine the likely mode of transmission and spread, and to optimize sampling methods. Under this Problem Statement, ARS scientists contribute epidemiological data that is critical for decision-making by State and Federal regulatory agencies, and for implementing disease control practices by growers and other scientists. Regulatory decisions are often made based on such studies, and ARS scientists have demonstrated the value of this data for delimiting specific diseases, including citrus canker.

The NP 303 Action Plan identified three anticipated Outputs that were expected to result from research addressing the needs expressed in Problem Statement 2C. In this problem statement, because of extensive overlap of these achievements among the anticipate Outputs, the accomplishments are not assigned to a specific Output.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. Robust statistical models to quantify relationships between disease levels and economic loss and analyzing impact to disease.
2. Mathematical models for disease forecasting/epidemic development of diseases with a user interface for growers.
3. Better sampling methods for pathogen dispersal.

PROBLEM STATEMENT 2C: SELECTED ACCOMPLISHMENTS

Packing house and field experiments on citrus canker leads to new national regulations and expansion of markets for citrus fruit. The termination of the Citrus Canker Eradication Program in Florida resulted in establishment of quarantines and fresh fruit marketing and shipping restrictions to protect canker-free areas from the introduction of the pathogen. Increased canker incidence throughout Florida resulted in decreasing number of citrus plantings that could be certified free of canker and available for interstate and international markets. This drastically limited citrus fresh markets and greatly increased tangible and intangible production costs, threatening industry survival. ARS scientists in Fort Pierce, Florida, conducted a stakeholder requested study that demonstrated fresh citrus fruit does not serve as a disease pathway for establishment of citrus canker in new areas. This resulted in revised regulations and expansion of national and international markets. The resulting research publication served as the

justification for APHIS to promulgate a new regulation that eliminated the requirement that fruit lots be inspected at the packing house, reducing industry costs by nearly $15 million a year.

Gottwald, T., Graham, J., Bock, C., Bonn, G., Civerolo, E., Irey, M., Leite, R., McCollum, G., Parker, P., Ramallo, J., Riley, T., Schubert, T., Stein, B., Taylor, E. 2009 The epidemiological significance of post-packinghouse survival of *Xanthomonas citri* subsp. *citri* for dissemination of Asiatic citrus canker via infected fruit. Crop Protection 28 (2009) 508–524

Model to quantify economic impact of stem rust disease in grass seed production. Stem rust is the most damaging disease affecting the production of cool-season grass seed crops, and U.S. growers spend millions of dollars each year to mitigate its damage. ARS scientists in Corvallis, Oregon, used data from 9 years of field experiments to derive a simple equation relating severity of rust disease to loss in seed yield. Information on damaging levels of disease is publicly available as an Internet decision aid that was produced in association with Oregon State University [See: http://uspest.org/cgi-bin/stemrust1.pl]. The decision tool computes epidemic severity from weather data and users' observations of disease, and has an action threshold based on the results of the yield-loss research that growers use to make management decisions. This will allow farmers to make appropriate economic decisions about the relative costs of management versus yield reduction for this critical production problem.

Pfender, W. 2009. A damage function for stem rust of perennial ryegrass seed crops. Phytopathology 99:498-505.

Multi-pest statewide, citrus exotic pest sampling method. State and Federal regulatory agencies needed methods to quickly survey entire states for newly introduced exotic pathogens. In 2009, ARS scientists in Fort Pierce, Florida, expanded a previous stochastic statewide stratified sampling/survey program for Huanglongbing/canker/citrus variegated chlorosis/*Citrus leprosis virus*, to include Black Spot, which was recently found for the first time in Florida. The survey is further adaptable if needed for future pest and pathogens. The revised multi-pest sampling/survey program is being utilized to determine the distribution of these diseases in commercial citrus throughout Florida and the survey program is adaptable to California and Texas statewide citrus industries.

Parnell, S., Riley, T., and Gottwald, T. R. 2007. Large-scale surveys for multiple pest species; the search for citrus canker and Huanglongbing in Florida. Phytopathology 96:S90.

Phytophthora ramorum and Sudden Oak Death. *Phytophthora ramorum*, the causal agent of Sudden Oak Death, has killed thousands of oaks in California and Oregon and is a threat to the vast oak forests of the eastern United States if the movement of the pathogen on nursery plants is not controlled. Research efforts at ARS deliver needed information that is incorporated into management practices of the natural, agricultural, and nursery communities. *P. ramorum* affects not only oak and tanoak trees, but has a usually wide host range that includes popular ornamental plants such as rhododendrons, viburnums, and camellias. ARS scientists are actively engaged in several aspects of research on *P. ramorum* aimed at determining the genetic and biological bases for infection and host range, which can be applied to management strategies. Following are selected research accomplishments:

Genetic identity and potential migration of P. ramorum isolates. ARS researchers from Corvallis, Oregeon, showed that the *P. ramorum* pathogen from California has a different genetic fingerprint from isolates found in the Pacific Northwest. Long distance migration of *P. ramorum* has occurred via the nursery trade, and shipments of host plants are known to have crossed the U.S.-Canada border. Results were compared to records compiled by APHIS on known shipments of infected plants, and these two sources of data were consistent with each other. Migration analysis of the group that is found in the United States, Canada, and European countries suggests that this group of isolates was introduced to North America from Europe. The results will help scientists and the nursery industry track the movement of this pathogen around the country and the world.

Goss, E.M., Larsen, M.M., Vercauteren, A., Werres, S., Heungens, K., Grunwald, N.J. 2011. *Phytophthora ramorum* detections in Canada: Evidence for migration within North America and from Europe. Phytopathology 101:166-171.

Temperature and moisture conditions for infection on Rhododendron. ARS scientists in Fort Detrick, Maryland, showed that *P. ramorum*-inducted disease occurred over a wide range of temperatures, although amounts of disease were minor at the temperature extremes. Moisture periods of 24 hours and 48 hours resulted in the greatest number of diseased leaves. The results help define conditions which lead to epidemic development and indicate that *P. ramorum* has the potential to become established in parts of the United States that are outside its current range. The information has been transferred to APHIS and the U.S. Forest Service scientists for use in the development of pest risk assessments and risk maps.

Browning, M.E., Englander, L., Tooley, P.W., Berner, D.K. 2008. Survival of *Phytophthora ramorum* hyphae following exposure to temperature extremes and various humidities. Mycologia 100:236-245.

Assay to quantify the soil phase of P. ramorum-infected nursery plants. *P. ramorum* has an active soil phase, which must be considered in regulatory and control efforts. ARS scientists in Beltsville, Maryland, developed an assay for quantifying numbers of pathogen spores present in runoff from infected plants. They determined the environmental conditions that favor root infection and production of inoculum, and related the number of spores in runoff to the resulting root infection. The assay was used to evaluate the risk associated with spores in runoff for containerized nursery stock and for native plant species. This research provided input for high-priority management issues posed by the Nursery Committee of the California Oak Mortality Task Force, a non-profit coalition that serves to coordinate research to minimize the impact and spread of *P. ramorum*. Results from runoff studies of native plant species will also help the U.S. Forest Service assess risk of *P. ramorum* to U.S. east coast ecosystems.

Shishkoff, N. 2011. A test system to quantify inoculum in runoff from Phytophthora ramorum-infected plant roots. Phytopathology 101:1457-1464.

Susceptibility of eastern forest species to P. ramorum. Twelve eastern U.S. forest species were screened against *P. ramorum* to see if they were susceptible. The pathogen caused stem and foliar lesions on all tree species tested. An important species in many

eastern forests, the chestnut oak (*Quercus prinus*), was found to be the most susceptible to both stem and foliar inoculation. This indicates the potential for *P. ramorum* to cause infection on eastern oak species were it to spread. Root-to-root spread was also demonstrated under flooded conditions. This is the first documentation of such spread and opens up a new area of inquiry and regulatory concern within *P. ramorum* epidemiology.

Tooley, P.W., Kyde, K.L. 2007. Susceptibility of some eastern forest species to *Phytophthora ramorum*. Plant Disease 91:435-438.

Virus survey of cherry trees in the national genetic resources. It has been challenging to conduct surveys of virus that infect stone fruits due to the lack of precise detection methods that can process large numbers of samples. ARS scientists in Beltsville, Maryland, developed or adapted real-time PCR assays for detecting 12 viruses infecting stone fruits. The assays developed were used in virus surveys to evaluate samples collected from cherry trees in the ARS National Plant Germplasm System repositories in Davis, California; Geneva, New York; and on the National Mall of the National Park Service and at the U.S. National Arboretum, both in Washington, D.C. Several viruses and different combinations and infection rates were detected at the four locations. Two viruses were common among trees sampled in Geneva, at the Arboretum, and on the National Mall. Sequence analyses of the real-time PCR products of these two viruses indicated that there were differences in the genetic variation. This survey helped to establish the virus infection status of cherry germplasm collections in the United States, and to validate protocols, which have been transferred to the APHIS quarantine program.

Li, R., Mock, R.G., Huang, Q., Abad, J., Hartung, J.S., Kinard, G.R. 2008. A reliable and inexpensive method of nucleic acid extraction for the PCR-based detection of diverse plant pathogens. Journal of Virological Methods 154:48-55.

Liming, L., Li, R., Mock, R.G., Kinard, G.R. 2011. Development of a polyprobe to detect six viroids of pome and stone fruits. Journal of Virological Methods 171:91-97.

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COMPONENT 3: Plant Disease Resistance

In addition to indigenous diseases, crop production in the United States is continuously threatened by the introduction of exotic plant diseases and emerging strains of domestic pathogens. To respond to these changing threats, plant breeders have incorporated genes for disease resistance into vulnerable crops. This process requires anticipating and recognizing plant disease threats, maintaining adequate germplasm stocks from which to draw disease resistance genes, and rapidly moving resistance genes from unimproved germplasm into agronomically desirable cultivars. Increasingly, providing information on the genetic basis of host resistance involves molecular genetic and genomic technologies, and new approaches in plant breeding devised from a better understanding of host-pathogen interactions.

This research component, especially Problem Statement 3B, complements and contributes to the plant breeding and germplasm research conducted in National Program 301, Plant Genetic Resources, Genomics, and Genetic Improvement. This partnership has lead to the development of more than 150 new varieties and germplasm lines (see Appendix 3 for a listing) conferring new disease resistance to economically important crops.

In Component 3, the research accomplishments address two broad problem statements as outlined in the NP303 Action Plan – 3A: Mechanisms of Plant Disease Resistance; and 3B: Disease Resistance in New Germplasm and Varieties.

PROBLEM STATEMENT 3A: *Mechanisms of Plant Disease Resistance*

Research conducted under this component increased understanding of crop resistance mechanisms and crop defense responses. The genetic and molecular bases of general defense mechanisms were clarified, as well as details of specific plant disease response mechanisms. ARS scientists characterized and annotated microbial genome sequence data and functional analysis of microbial genomes to understand adaptive features essential for pathogenesis.

Teams of ARS scientists and their collaborators made significant advances during the past 5 years in phenotyping and genotyping germplasm for host resistance to pathogens. In particular, disease resistance quantitative trait loci (QTL) identification and mapping, coupled with genome-wide association studies have resulted in greater understanding of the genetic basis of complex traits. As a result, new, disease-resistant germplasm and varieties can be more accurately and quickly identified. This research bridges basic knowledge of how disease resistance operates in plants with the development of disease resistant crops, as outlined in Problem Statement 3B.

The Action Plan identified three anticipated Outputs that were expected from ARS research addressing the needs expressed in Problem Statement 3A. Accomplishments that illustrate those products for this problem statement follow the list of anticipated Outputs.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. Expanded genetic and genomic resources for crops, their pathogens, and other microbes needed to develop new strategies to protect crops from disease.

2. Discovery of the underlying pathogenic mechanisms essential for the initiation, establishment, and spread of plant disease.
3. Increased knowledge of disease-resistance mechanisms in crop plants.

PROBLEM STATEMENT 3A: SELECTED ACCOMPLISHMENTS

Output 1: *Expanded genetic and genomic resources for crops, their pathogens, and other microbes needed to develop new strategies to protect crops from disease.*

Race- specific resistance to rice blast decoded. Blast disease is a major yield limitation for rice production worldwide. ARS researchers in Stuttgart, Arkansas, discovered that in 15 rice cultivars one domain of the Pi-km gene is the same across cultivars, whereas another domain that is highly variable is associated with resistance to different races of the blast pathogen. DNA markers specific for this domain were developed and have been exploited by breeders to stack resistance genes in new cultivars, thus reducing the need for applying fungicides. The new markers will simplify the introgression of the valuable blast resistance associated with the complex Pi-k locus into rice cultivars.

Costanzo, S. and Jia, Y. 2010. Sequence variation at the rice blast resistance gene *Pi-km* locus: Implications for the development of allele specific markers. Plant Science 176: 523-530.

Identification of multiple disease resistance in maize. Southern corn leaf blight (caused by the fungus *Cochliobolus heterostrophus*; See Figure 8), northern corn leaf blight (caused by the fungus *Setosphaeria turcica*), and gray leaf spot (caused by the fungus *Cercospora zeae-maydis*) are among the most damaging diseases of maize worldwide. ARS researchers in Raleigh, North Carolina; Ithaca, New York; and Columbia, Missouri, in collaboration with university colleagues and industry partners, developed a statistical methodology for combining QTL mapping data from recombinant inbred line populations, with the subsequent near-isogenic lines derived from the recombinant inbred lines. This resulted in relatively precise localization of the QTL for southern leaf blight resistance (within less than 0.5cM interval). This new technology was used to assess 300 diverse maize lines for resistance to southern leaf blight and two other maize diseases (gray leaf spot and northern leaf blight), and to determine that the glutathione S-transferase gene was associated with resistance to all three maize diseases, and can serve as a useful marker for breeding and further modes of action studies**.**

Kump, K., Bradbury, P., Buckler, E., Belcher, A., Oropeza-Rosas, M., Wisser, R., Zwonitzer, J., Kresovich, S., McMullen, M., Ware, D., Balint-Kurti, P., and Holland, J. 2011. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nature Genetics 43:163-168.

Kump, K., Holland, J., Jung, M., Wolters, P., Balint-Kurti, P. 2010. Joint analysis of near isogenic and recombinant inbred line populations yields precise positional estimates for QTL. Plant Genome 3:142–153.

Wisser, R., Kolkman, J., Patzoldt, M., Holland, J., Yu, J., Krakowsky, M., Nelson, R., and Balint-Kurti, P. 2011. Multivariate analysis of resistances to three diseases in maize suggests a pleiotropic genetic basis and implicates a glutathione S-transferase as a multiple disease resistance gene. Proceedings of the National Academy of Sciences 108:7339-7344.

Figure 6: Leaves from four maize genotypes infected with southern leaf blight. The commonly-used genetic stock B73 is shown at the top. Each of the other lines is near-isogenic (~ 95 percent genetically identical) to B73, but each has a different resistance QTL introgressed into it. *Photos by Peter Balint-Kurti/ARS.*

Identification and mapping of new sources of resistance to aflatoxin accumulation in maize. Aflatoxins are highly carcinogenic toxins produced by several molds before harvest and during storage in corn, peanuts, cottonseed, tree nuts, and other crops. ARS researchers and their collaborators are conducting research with two complementary approaches to control aflatoxin contamination in crops. One is 'competitive exclusion' – applying benign strains to impede colonization by harmful strains of fungi that contaminate crops with aflatoxin (described in Component 4) – and the other is developing germplasm that resists buildup of aflatoxin. ARS researchers from Starkville, Mississippi, identified QTLs on most maize chromosomes for resistance of aflatoxin accumulation. Individual QTLs explained from 1 percent up to 12 percent of the phenotypic variation of the trait, and markers linked to these QTLs were developed. This confirms that this source of resistance to aflatoxin accumulation in maize is novel, and effective markers are available for use in breeding for resistance to aflatoxin.

Warburton, M.L., Brooks, T.D., Krakowsky, M.D., Shan, X., Windham, G.L., Williams, W.P. 2009. Identification and mapping of new sources of resistance to aflatoxin accumulation in maize. Crop Science 49:1403-1408.

Williams, W.P., Windham, G.L. 2006. Registration of maize germplasm line Mp717. Crop Science 46:1407-1408

Genetic markers for evaluating powdery mildew resistance. ARS researchers in Parlier, California, and their cooperators discovered a genetic marker for a broad spectrum powdery mildew resistance locus in grape, and also documented a powdery mildew resistance mechanism not previously described in the Vitaceae or elsewhere. This dominantly inherited resistance prevents hyphal emergence by the causal agent, *Erisyphe necator*, and is non-race specific and tissue-independent. In addition to its role in breeding for durable resistance, this gene (*Ren4*) will provide mechanistic insights into the early events that enable powdery mildew infection, enabling more efficient evaluation of breeding material to develop future cultivars, requiring fewer pesticide applications.

Ramming, D.W., Gabler, F., Smilanick, J.L., Cadle Davidson, M., Barba, P., Consolie, N.H., Mahanil, S., Cadle Davidson, L.E. 2011. A single dominant locus Ren4 confers non-race-specific penetration resistance to grapevine powdery mildew. Phytopathology 101(4):502-508.

Release of sugarbeet germplasm with resistance to new strains of Beet necrotic yellow vein virus. Rhizomania, one of the most devastating diseases of sugarbeet, is caused by *Beet necrotic yellow vein virus* vectored by the fungus *Polymyxa betae* in soil. Since 2003, the Rz1 resistance gene has been compromised by new resistance-breaking strains of *Beet necrotic yellow vein virus*. Sugarbeet germplasm with resistance to the virus is needed for sugarbeet growers to maintain production in contaminated soils. ARS scientists in Salinas, California, developed and released sugarbeet germplasm lines with resistance to the new strains. Resistance was introgressed from wild beet *Beta vulgaris* subsp. *maritima* into breeding lines. These germplasm resources represent ongoing efforts to combine multiple disease resistance with high productivity, and to enhance sugarbeet breeding populations with genes from wild beet accessions. These germplasm lines will give the sugarbeet seed industry an additional source of genetic resistance to rhizomania.

Lewellen, RT and Liu, H-Y. 2008. Notice of release of C812-41 and C812-41CMS sugarbeet germplasms with resistance to resistance-breaking strains of Beet necrotic yellow vein virus. Official Germplasm Release, signed 05/28/2008.

Output 2: ***Discovery of the underlying pathogenic mechanisms essential for the initiation, establishment, and spread of plant disease.***

Defining the susceptibility response of a necrotrophic pathogen of wheat, Stagonospora nodorum. Stagonospora nodorum blotch is a worldwide, destructive foliar disease of both common wheat and durum wheat. Many plant necrotrophic pathogens, such as *Stagonospora nodorum*, produce effectors (host-selective toxins) that, when recognized by specific genes in

wheat, cause cell death allowing the pathogen to grow and complete their life cycle. These effector proteins can suppress inherent disease resistance responses of plants to varying degrees.

ARS researchers in Fargo, North Dakota, and Manhattan, Kansas, with their collaborators, identified and cloned host-selective toxins that confer susceptibility of adult wheat plants to this disease. This discovery confirmed that the pathosystem is mainly based on an inverse gene-for-gene process between the host and toxins (Figure 9). This work furthers scientific understanding of susceptibility mechanisms to plant pathogens, and strengthens the concept that toxin sensitivity genes should be eliminated from modern wheat varieties to obtain enhanced resistance to diseases caused by necrotrophic pathogens.

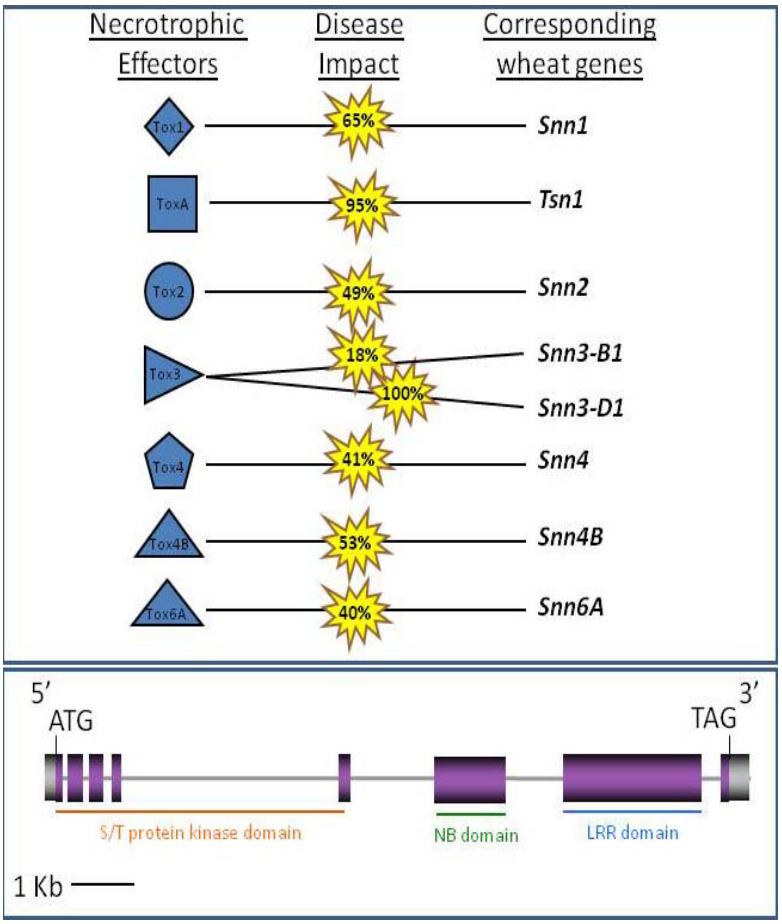

Figure 7: Top panel: Necrotrophic effectors (left) produced by the wheat pathogen *Stagonospora nodorum* and the corresponding dominant sensitivity/susceptibility genes in wheat (right). The necrotrophic effector – host gene interactions account for 18 percent to 100 percent of the disease interaction. Bottom panel: Molecular structure of the *Tsn1* gene. *Tsn1* harbors disease resistance gene-like serine/threonine protein kinase, nucleotide binding, and leucine-rich repeat domains, but in this case the gene confers susceptibility to the necrotrophic pathogen *Stagonospora nodorum. Graphic by Tim Fresen/ARS.*

Chu, C.G., Faris, J.D., Xu, S.S., Friesen, T.L. 2010. Genetic analysis of disease susceptibility caused by compatible *Tsn1-SnToxA* and *Snn1-SnTox1* interactions in the wheat-*Stagonospora nodorum* pathosystem. Theoretical and Applied Genetics 120:1451-1459.

Faris, J.D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J.P., Meinhardt, S.W., Rasmussen, J.B., Xu, S.S., Oliver, R.P., Simons, K.J., Friesen, T.L. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to Nencrotrophic pathogens. Proceedings of the National Academy of Sciences 107:13544-13549.

Faris, J.D., Zhang, Z., Rasmussen, J.B., Friesen, T.L. 2011. Variable expression of the *stagonospora nodorum* effector SnToxA among isolates is correlated with levels of disease susceptibility in wheat. Molecular Plant-Microbe Interactions 24:1419-1426.

Output 3: ***Increased knowledge of disease-resistance mechanisms in crop plants.***

New knowledge of wheat pathogen biology and resistance for development of stripe rust resistant wheat. Since 2000, wheat stripe rust has re-emerged as an important disease in the United States, particularly in the southern Great Plains and southeastern United States. ARS researchers in Pullman, Washington; Fargo, North Dakota; and Raleigh, North Carolina, with their university colleagues discovered four QTLs for resistance to the new races of the stripe rust fungus, *Puccinia striiformis*. These QTLs represent the first, highly effective QTLs identified to the newly re-emerged races of stripe rust.

Coram, T.E., Huang, X, Zhan, G. Settles, M.L., Chen, X. 2010. Meta-analysis of transcripts associated with race-specific resistance to stripe rust in wheat demonstrates common induction of blue copper-binding protein, heat-stress transcription factor, pathogen-induced WIR1A protein, and ent-kaurene synthase transcripts. Functional Integrative Genomics 10:383-392.

Lowe, I. Jankuloski, L., Chao, S., Chen, X., See, D., Dubcovsky, J. 2011. Mapping and validation of QTL which confer partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. Theoretical and Applied Genetics 123:143-157.

A Potato virus Y resistant potato developed using intragenic technology. *Potato virus Y* is the most important disease problem for the seed potato industry. Emerging *Potato virus Y* tuber necrotic strains threaten the quality of the commercial crop unless effective control strategies are developed. ARS researchers in Ithaca, New York, and Aberdeen, Idaho, in collaboration with university colleagues, developed and field-tested an intragenic potato line that is resistant to multiple strains of the virus. Natural mutations in the plant eIF4E translation factor confer resistance to potyviruses in many plant species, but not potato. Using knowledge about eIF4E resistance genes in other solanaceous plants, the scientists modified the potato eIF4E gene to resemble resistance alleles from pepper. When the modified potato genes were over-expressed in potato cultivar Russet Burbank, the plants were unable to support the accumulation or systemic movement of several different strains of *Potato virus Y*. These resistant plants grew similar to untransformed controls and produced similar numbers of tubers. The virus was not detected in plants sprouted from any of the tubers from the inoculated resistant plants. This strategy will be applicable for engineering *Potato virus Y* resistance in any potato cultivar, as well as resistance in other crops to a number of economically important viruses. The intragenic strategy, whereby the transgene is derived from the target crop itself, lessens concerns associated with transgenic crops.

Cavatorta, J., Perez, K., Gray, S.M., Vanek, J., Yeam, I., Jahn, M. 2011. Engineering virus resistance using a modified potato gene. Plant Biotechnology Journal DOI: 10.1111/j.1467-7652.2011.00622.x.

Genetic resistance to Cucurbit leaf crumple virus in melon. *Cucurbit leaf crumple virus*, transmitted by *Bemisia tabaci* biotype B, is a Begomovirus common in fall melons in the American desert southwest that renders plants non-productive. ARS scientists in Salinas, California, found that melon breeding line MR-1 and six germplasm accessions that were partially resistant to the virus in naturally infected field tests and controlled inoculation

greenhouse tests. One accession was completely resistant in two greenhouse tests, while other lines exhibited partial resistance as confirmed by PCR amplification of viral DNA. Genetic resistance to *Cucurbit leaf crumple virus* in melon involves a single recessive gene, providing an important means of managing this virus in an environment where other methods only slow the disease because of heavy vector pressure. It also avoids the need for heavy use of insecticides for vector control of Begomoviruses.

McCreight, J.D., Liu, H.Y., Turini, T.A. 2008. Genetic resistance to cucurbit leaf crumple virus in melon. 2008. HortScience 43: 122-126.

PROBLEM STATEMENT 3B: ***Disease Resistance in New Germplasm and Varieties.***

ARS scientists have documented and responded to changes in pathogen populations by identifying new crop host genes for resistance to these pathogens, and incorporating them into valuable germplasm or commercial cultivars for release.

Significant advances in identifying and deploying host resistance were achieved for wheat stem rust, soybean rust, and diseases of many specialty crops. Resistant genes were identified from germplasm in ARS plant collections and were characterized; host-plant relationships were elucidated; and rapid ways to transfer genes into adapted cultivars through molecular marker technologies were developed. These efforts often yielded improved germplasm for further development by breeders in the public and private sectors. The potentially severe effects of soybean rust, for example, were averted in part by the accomplishments of NP 303 scientists. ARS researchers have erected defenses against emerging highly virulent races of wheat stem rust, such as Ug99, that have not yet arrived in the United States.

During the past 5 years, ARS scientists developed new disease-resistant germplasm and varieties in many crops. Novel methodologies were devised for introgressing genes. Outstanding accomplishments were achieved in DNA marker development, mapping, and sequencing of various pathogen populations and crop resistance genes. Where breeders lacked genetic diversity, for example in the case of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 attacking cotton, new host resistance diversity was created by NP 303 scientists.

The Action Plan identified four anticipated Outputs that were expected from ARS research addressing the needs expressed in Problem Statement 3B. The accomplishment examples in this section following the list of anticipated Outputs demonstrate the key role of component 3B in relation to the entire NP 303 program.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. Monitor genetic changes in critical pathogen populations by pathogen surveys.
2. Characterize germplasm collections to identify new genes for disease resistance.
3. Develop molecular markers that facilitate plant breeding for disease resistance.
4. Discover and apply more efficient methods for incorporating disease resistance genes into crop plants.

PROBLEM STATEMENT 3B: SELECTED ACCOMPLISHMENTS

Output 1: *Monitor genetic changes in critical pathogen populations by pathogen surveys.*

New virulence in stem rust races from Kenya. Although the emerging threats from highly virulent stem rust of wheat and barley are often discussed as caused by a single pathogenic strain of *Puccinia graminis* f. sp. *tritici*, Ug99, the current epidemics are caused by a family of strains, including Ug99 and close relatives. Stem rust resistance, provided by the Sr24 gene that is effective against most stem rust races, was widely used in breeding, and accounted for 40-60 percent of the Ug99 resistance that has been-incorporated into various classes of wheat from the United States, CIMMYT, South Africa, and other major wheat production regions. ARS researchers in St. Paul, Minnesota, discovered a strain of the stem rust pathogen from East Africa closely related to Ug99 that could overcome the resistance provided by the Sr24 gene. Another closely related strain was identified which could overcome the Sr36 gene, which is present in many eastern U.S. soft winter wheats. The occurrence of pathogen virulence against three widely used host resistance genes (Sr24, Sr31, and Sr36) substantially increases the vulnerability of wheat to stem rust worldwide because of the widespread use in breeding of these genes. Discovery of these new strains prompted quick action in seeking control strategies. The occurrence of virulence to Sr24, Sr31, and Sr36 in East Africa has highlighted the need for developing and deploying cultivars with combinations of effective resistance genes to enhance durability.

Jin, Y., Szabo, L.J., Pretorious, Z., Singh, R., Fetch, Jr., T. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Disease 92:923-926.

Jin, Y., Szabo, L.J., Rouse, M.N., Fetch, Jr., T., Pretorius, Z.A., Wanyera, R., Njau, P. 2009. Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. Plant Disease 93:367-370.

Pathogenic variation of the Asian soybean rust pathogen. Asian soybean rust, caused by *Phakopsora pachyrhizi*, is an important disease in many soybean producing countries. ARS scientists at Urbana, Illinois, and researchers at the University of Ibadan in Nigeria examined the geographical distribution and genetic variation of Asian soybean rust isolates in Nigeria to extrapolate potential distributions across ecological zones in the United States. The researchers surveyed fields in three agro-ecological zones, collected 116 purified isolates, and established them on detached soybean leaves. Based on rates of reproduction of the fungi on selected soybean lines, the isolates were separated into seven groups. This was the first report grouping soybean rust isolates based on their abilities to reproduce on many different soybean lines. Soybean researchers in the United States and other countries applied these insights to determine the occurrence and distribution of rust based on disease surveys, and to determine the variability of the pathogen relative to host resistance.

Twizeyimana, M., Ojiambo, P.S., Haudenshield, J.S., Caetano-Anolles, G., Pedley, K.F., Bandyopadhyay, R., Hartman, G.L. 2009. Pathogenic variation of *Phakopsora pachyrhizi* infecting soybean in Nigeria. Phytopathology 99(4):353-361.

Differential responses of resistant soybean genotypes to isolates of Phakopsora pachyrhizi. Since detection of Asian soybean rust, caused by the fungus *Phakopsora pachyrhizi*, in the United States, several new sources of resistance to *P. pachyrhizi* have been identified in soybean. But, how well the available resistance sources protect soybean plants from infection by other U.S. and international soybean rust isolates was unknown. ARS researchers in Urbana, Illinois, compared disease responses of 20 soybean lines after inoculation with 10 *P. pachyrhizi* isolates from different geographic and temporal regions selected from field trials in Paraguay, Vietnam, Taiwan, and India. The resistant soybean lines selected from Paraguay and Vietnam varied considerably in their responses to the pathogen isolates. *P. pachyrhizi* isolates from Taiwan produced the greatest number of susceptible reactions, whereas isolates from India produced the greatest number of resistant reactions on soybean. This research identified soybean lines that may be novel sources of resistance to Asian soybean rust. Although a standard set of soybean pathogen differentials is not finalized, these results provide a basis for an effective set of soybean genotypes to distinguish among different Asian soybean rust pathotypes based on virulence patterns.

Pham, T.A., Miles, M.R., Frederick, R.D., Hill, C.B., Hartman, G.L. 2009. Differential responses of resistant soybean genotypes to ten isolates of *Phakopsora pachyrhizi*. Plant Disease 93:224-228.

Output 2: **Characterize germplasm collections to identify new genes for disease resistance.**

***Effective crown rust resistance found in a wild Avena species*.** Crown rust caused by *Puccinia coronata* is the most destructive disease of oat worldwide. As new, resistant oat varieties are developed, they quickly succumb to new corresponding races of the crown rust pathogen. The wild tetraploid slender oat, *Avena barbata*, has been a source of powdery mildew and stem rust resistance in cultivated oat, but has largely been unexploited for crown rust resistance. ARS scientists in St. Paul, Minnesota, screened 359 accessions of *A. barbata* from the USDA National Small Grains Collection for resistance to a diverse population of *P. coronate*, which contained virulence to all the described crown rust resistance genes in cultivated oat. Of these accessions, 48 were identified as resistant to the pathogen population screened. These lines represent a new, unexploited pool of novel resistance genes available to breeders for oat improvement.

Carson, M.L. 2009. Broad spectrum resistance to crown rust, *Puccinia coronata* f. sp. *avenae*, in U.S. accessions of the tetraploid slender oat, *Avena barbata*. Plant Disease. 93:363-366.

Peach-almond hybrids are non-hosts of the almond leaf scorch disease pathogen. California's 700,000+ acres of almonds are susceptible to almond leaf scorch, a disease caused by the insect-transmitted bacterium *Xylella fastidiosa*. Currently there are no effective management techniques that protect almond trees from infection by *X. fastidiosa*. Growers must decide to replace or keep almond leaf scorch-diseased trees that serve as sources of inoculum for healthy trees. ARS researchers in Parlier, California, developed a new peach-almond hybrid rootstock and demonstrated that *X. fastidiosa* could not survive in this rootstock. Planting the peach-almond hybrid rootstock in commercial orchards may reduce incidence of almond leaf scorch disease by eliminating one possible source of the bacterium. Peach-almond rootstocks also

provide superior anchorage and tree vigor, and are in widespread use in commercial orchards in California.

Ledbetter, C. A., and Rogers, E. E. 2009. Differential susceptibility of *Prunus* germplasm (Subgenus Amygdalus) to a California isolate of *Xylella fastidiosa*. HortScience 44:1928-1931.

Second source of resistance to reniform nematode identified for cotton. In 2009, U.S. cotton producers lost an estimated $60 million in damage to the reniform nematode. ARS researchers in College Station, Texas, and their university colleagues and industry partners, mapped genes for resistance to reniform nematode onto chromosome 11 from the wild cotton species *Gossypium longicalyx*. A second source of nematode resistance in *Gossypium barbadense*, GB713, provides an excellent additional source of resistance to root knot nematode. Because this resistance source is from *G. barbadense*, a cultivated tetraploidcotton, it should be more readily transferrable to upland cotton (*G. hirsutum*). This research also identified markers for selecting root knot nematode resistance genes. Advanced cotton lines carrying this marker are being evaluated in the field.

Gutierrez, O.A., Robinson, A.F., Jenkins, J.N., McCarty, J.C., Wubben, M.J., Callahan, F.E., Nichols, R.L. 2011. Identification of QTL regions and SSR markers associated with resistance to reniform nematode in *Gossypium barbadense* L. accession GB713. Theoretical and Applied Genetics 122(2):271-280.

Grain mold-resistant sorghum germplasm. Identifying new grain mold-resistant sorghum germplasm for exploitation by breeders is the most desirable approach to successful management of this disease. ARS researchers at College Station, Texas, working with scientists in Senegal and university collaborators, identified six sorghum lines from West African sources that are highly resistant to grain mold strains that occur in Senegal and Texas. These new grain mold-resistant sorghum germplasm are being exploited by sorghum breeders to develop new disease-resistant sorghum.

Prom, L.K., Erpelding, J.E. 2009. New sources of grain mold resistance among accessions from Sudan. Tropical and Subtropical Agroecosystems 10:457-463.

Prom, L.K., Isakeit, T., Perumal, R., Erpelding, J.E., Rooney, W.L., Magill, C.W. 2011. Evaluation of the Ugandan sorghum accessions for grain mold and anthracnose resistance. Crop Protection Journal 30(5):566-571.

Ug99-resistant and high quality wheat varieties. ARS researchers in Lincoln, Nebraska, and Raleigh, North Carolina, have released, or contributed to the release, of multiple new wheat varieties with disease resistance and new end-product qualities. These include two varieties with the first Ug99 resistance for wheat adapted to the United States – Nueast (2009), a hard red winter wheat, and Appalachian White (2009), a hard white wheat, both bred for the southeastern United States. Other releases include Mace (2008), a hard, red winter wheat cultivar for the Midwest with resistance to Ug99 and wheat streak mosaic virus; and Anton (2008), a hard white wheat with low polyphenoxidase activity that yields superior flour for Asian noodles. These cultivars are currently in production.

Graybosch, R.A., Peterson, C.J., Baenziger, P.S., Baltensperger, D.D., Nelson, L.A., Jin, Y., Kolmer, J.A., Seabourn, B.W., French, R.C., Hein, G.L. 2009. Registration of 'Mace' hard red winter wheat. Journal of Plant Registrations 3:51-56.

Graybosch, R.A., Peterson, C.J., Baenziger, P.S., Baltensperger, D.B., Nelson, L., Jin, Y., Kolmer, J.A., Seabourn, B.W., Beecher, B.S. 2011. Registration of Anton hard white winter wheat. Journal of Plant Registrations 5. DOI: 10.3198/jpr2010.08.0481crc.

Output 3: ***Develop molecular markers that facilitate plant breeding for disease resistance.***

Genetic markers for table grape and raisin varieties with high fruit quality and Pierce's disease resistance. Pierce's disease, caused by the bacterium *Xylella fastidiosa,* damages grapevines in California, across the southern United States, and into South America. Introduction of resistance to Pierce's disease from wild grape species into table grape and raisin varieties usually results in small berries and poor fruit quality. ARS scientists in Parlier, California, incorporated Pierce's disease resistance into elite wine, table, and raisin grape genetic backgrounds via repeated back-crossing, accelerated by reliable PCR-based markers. Pierce's disease resistance from *Vitis arizonica* was incorporated into elite grape breeding lines while maintaining high quality fruits. The current advanced selections will provide growers with high quality resistant cultivars. The research modernized the introgression procedure for this disease, incorporating resistant germplasm without prolonged field or greenhouse studies.

Riaz, S., Tenscher, A.C., Graziani, R., Krivanek, A.F., Ramming, D.W., Walker, M. 2009. Using marker-assisted selection to breed Pierce's disease-resistant grapes. American Journal of Enology and Viticulture 60(2):199-207.

Fine-mapping root knot nematode resistance in cotton. Identifying DNA markers tightly linked to resistance genes is a key step toward marker-assisted selection in breeding cotton for nematode resistance. ARS scientists in Tifton, Georgia, and their university colleagues precisely defined the location of a QTL for root knot nematode resistance in cotton to a 3.6 cM region flanked by two DNA markers. These results provide a critical tool for marker-assisted selection of root knot nematode resistance in cotton and also will enable map-based isolation of the nematode resistance gene. Public and private plant breeders have already applied these markers in breeding for root knot nematode resistance in cotton**.**

Shen, X., He, Y., Lubbers, E.L., Davis, R.F., Nichols, R.L., Chee, P.W. 2010. Fine mapping QMi-C11 a major QTL controlling root-knot nematodes resistance in upland cotton. Theoretical and Applied Genetics 121:1623-1631.

Durable root-knot nematode resistance in peanut. Three root knot nematode species parasitize peanut in the United States – *Meloidogyne arenaria, M. hapla,* and *M. javanica*. Some commercial peanut cultivars are resistant to *M. arenaria* and *M. javanica,* but no peanut cultivar or released germplasm is resistant to *M. hapla*. Sources of resistance to all three nematodes are needed for broad and durable resistance to root-knot nematodes. ARS scientists at Tifton, Georgia, and their university collaborators tested cultivars and breeding lines for resistance to the three nematode species and for molecular markers linked to the resistance genes. Breeding lines were identified with high levels of resistance to *M. hapla*, with two lines also having resistance

to *M. arenaria* and *M. javanica.* Peanut breeders have incorporated these lines into programs for developing peanut cultivars with resistance to multiple species of root-knot nematodes.

Dong, W. B., Holbrook, C. C., Timper, P., Brenneman, T. B., Chu, Y., and Ozias-Akins, P. 2008. Resistance in peanut cultivars and breeding lines to three root-knot nematode species. Plant Disease 92:631-638.

New markers and sources of Ug99 wheat stem rust resistance identifed. In a cooperative project with CIMMYT and the Kenyan Agricultural Research Institute in Njoro, ARS researchers in St. Paul, Minnesota, and Raleigh, North Carolina, evaluated Ug99 resistance levels in Kenya for 21,000 lines from U.S. wheat and barley breeders from 28 universities, 11 private sector companies, and ARS genebanks. New sources of Ug99 resistance were identified in wild wheat relatives, land races, and other accessions from the ARS National Small Grains Collection in Aberdeen, Idaho. Molecular markers that tag the stem rust resistance genes *Sr24, Sr26, Sr36,* and *Sr1RS* Amigo and that confer resistance to the virulent Ug99 wheat stem rust strain were identified. A collection of 776 cultivars and breeding lines of wheat from all growing regions of the United States was analyzed genetically to determine frequencies of these genes in U.S. wheat germplasm. ARS researchers also identified Ug99 resistance in the A-genome diploid relatives of wheat after screening 1,061 accessions of *Triticum monococcum* and 214 accessions of *T. urartu* against race Ug99. The researchers found that many of the accessions (79 percent of *T. monococcum* and 93 percent of *T. urartu*) were resistant to Ug99. These are previously uncharacterized resistance genes to Ug99 and provide critically needed new sources of resistance in wheat. This information is enabling the introgression of new sources of Ug99 resistance and development of resistance gene pyramids for more durable stem rust resistance.

Bonman, J.M., Bockelman, H.E., Jin, Y., Hijmans, R.J., Gironella, A. 2007. Geographic distribution of stem rust resistance in eheat landraces. Crop Science 47:1955-1963.

Olson, E.L., Brown Guedira, G.L., Marshall, D.S., Jin, Y., Mergoum, M., Lowe, I., Dubcovsky, J. 2010. Genotyping of U.S. wheat germplasm for presence of stem rust resistance genes *Sr24*, *Sr36* and *Sr1RS* Amigo. Crop Science 50:668-675.

Rouse, M., Jin, Y. 2011. Stem rust resistance in A-genome diploid relatives of wheat. Plant Disease 95:941-944.

Output 4: ***Discover and apply more efficient methods for incorporating disease resistance genes into crop plants.***

Stripe rust wheat gene identification and mapping. Cultivars with genetic resistance to diseases are an effective, economical, and environmentally friendly approach for control of stripe rust in wheat. Only few available genes were effective against all races of the stripe rust pathogen. Applying a new batched bulk molecular marker approach, ARS scientists in Pullman, Washington, identified more than 20 different genes conferring effective resistance to stripe rust in 38 wheat germplasm lines, and developed populations to map these genes. One of the genes was effectively mapped to a wheat chromosome – the long arm of chromosome 3D – and is being selected by wheat breeders. The genes discovered, along with the mapping populations, are also the basis for marker-assisted breeding strategies for stripe rust resistance.

Li, Q., Chen, X., Wang, M., Jing, J. 2010. Yr45, A new wheat gene for stripe rust resistance mapped on the long arm of chromosome 3D. Theoretical and Applied Genetics 122:189-197.

Discovery of Rhizoctonia and Pythium tolerance in wheat. All varieties of wheat are susceptible to the soilborne pathogens *Rhizoctonia* and *Pythium,* and genetic resistance to these pathogens has been elusive. ARS scientists in Pullman, Washington, in collaboration with university scientists, developed a wheat genotype, Scarlet-Rz1, using chemical mutagenesis that is tolerant to the four species of Rhizoctonia and Pythium tested. Scarlet-Rz1 significantly lowers disease severity and reduces root loss when infected with the pathogens as compared to native Scarlet. This novel, stable source of genetic resistance to these hard-to-control soilborne pathogens offers a sustainable means of combating *Rhizoctonia* and *Pythium*, and provides a new source of resistance for wheat improvement programs. Three types of perennial wheat were also tolerant to these root diseases. Plants exhibited lower disease severity ratings and sustained less root damage as compared to annual wheat lines that were infected with one or more pathogen. Wheat breeding programs have been using these newly developed sources to develop resistant varieties.

Okubara, P.A., Jones, S.S. 2011. Seedling tolerance to *Rhizoctonia* and *Pythium* in wheat chromosome group 4 addition lines from *Thinopyrum* spp. Can. J. Plant Pathol. 33(3): 415-422.

Okubara, P.A., Steber, C.M., Demacon, V.L., Walter, N., Paulitz, T.C., Kidwell, K.K. EMS-treated hexaploid wheat genotype Scarlet has enhanced tolerance to the soilborne necrotrophic pathogens *Rhizoctonia solani* AG-8 and *R. oryzae*. 2009. Theor. Appl. Genet. 119: 293-303.

Stacking genes for resistance to both root knot and reniform nematodes in cotton. Root knot and reniform nematodes cause losses of almost 5 percent of the U.S. cotton crop. Commercial cotton cultivars have limited resistance to the root knot nematode, and none are resistant to the reniform nematode. ARS scientists in College Station, Texas, in collaboration with university researchers, applied marker-assisted breeding to combine the rkn-1 gene from Acala Nemex cotton (which confers resistance to the root knot nematode) with the Ren gene from wild *Gossypium longicalyx* (which confers resistance to the reniform nematode). The resulting lines are resistant to both nematodes. The molecular markers are readily available to commercial breeders to introduce these combined resistance traits into commercial cottons, and accelerate the release of new resistant cotton germplasm.

Dighe, N.D., Robinson, A.F., Bell, A.A., Menz, M.A., Cantrell, R.G., Stelly, D.M. 2009. Linkage mapping of *Gossypium longicalyx* resistance to reniform nematode during introgression into cotton *Gossypium hirsutum*. Crop Science 49:1151-1164.

SPECIAL RESEARCH INITIATIVES AND RELATED PROGRAMS IN NP303

Special research initiatives are one mechanism whereby ARS provides leadership and national coordination for plant disease research. Bringing together Federal, State, industry, and, sometimes, international scientists to identify knowledge gaps and develop action plans with

common goals and objectives, these ARS-led activities optimize resources and avoid duplication of effort. Scientists in NP 303 have been leaders of initiatives in Fusarium head blight, Sclerotinia, Asian soybean rust, and Ug99. These integrated research programs combine research, communication, and outreach to combat new virulent pathogens that threaten sustainable production of key crops. Crop germplasm has been evaluated for disease vulnerability, diagnostic technologies and disease management systems developed, and new varieties with genetic protection against disease have been released. Strategic deployments of resources and technologies have minimized the impact of new and emerging pathogen races. ARS has coordinated research among Federal, State, and international organizations via steering committees that develop comprehensive and optimized research plans for most effectively employing research funds.

The relevance of the research plan is assured by the central role that crop growers and/or processors play in the initiatives' development. The structure of the Action Plans for these initiatives provides both a program and scientific focus to ensure that the research, extension, and outreach produce outcomes in an effective and timely manner. The strategic goals of the Action Plans span the programmatic range of establishing national communications networks, demonstrating effective disease management systems, understanding host-pathogen interactions, developing enhanced germplasm, improving knowledge of pathogen biology, and developing decision models to guide the implementation of control measures. A significant part of these initiatives are aimed at developing disease-resistant cultivars or germplasm, and thus are coordinated with Component 3 of NP 303. Selected accomplishments from ARS research initiatives are summarized below.

WHEAT AND BARLEY SCAB INITIATIVE

Fusarium Head Blight (also called scab) results in direct crop yield losses as well as losses in quality due to contamination of grain with the mycotoxin deoxnivalenol (DON; also called vomitoxin). Combined losses to all steps in the food system are difficult to estimate, but the bill at the farm-gate alone is estimated to exceed $4 billion from 1991-2000. The U.S. Wheat and Barley Scab Initiative (USWBSI; www.scabusa.org), formed in 1997, has the mission of developing as quickly as possible effective control measures that minimize the threat of Fusarium head blight, including the reduction of mycotoxins, to the producers, processors, and consumers of wheat and barley. In addition to the research accomplishments listed below, the USWBSI developed and provides publically available tools for research and disease management, including ScabSmart, Scab alerts by smartphone or e-mail, Risk Assessment tools, information on resistance and susceptible cultivars, databases on host resistance, and regional management information. Growers have avoided substantial losses to scab by applying these Initiative-funded prediction models, management advisories, and by avoiding vulnerable grain varieties.

The USWBSI provides DON testing for researchers and 67 wheat and barley breeders in 21 states. The availability of data on DON levels in breeding lines resulted in the release of numerous cultivars with increased resistance to DON development. The USWBSI is responsible for the increase in acreage of wheat and barley planted to resistant cultivars and the concomitant decline in DON levels in U.S. grain. In durum wheat alone, DON declined 32 percent over the past 10

years. The USWBSI has leveraged its resources, e.g., funding for expressed sequence tags (ESTs) of *Fusarium graminearum* provided data to secure other funding for the entire genome. As a result, this was one of the first fungal pathogens fully sequenced. The USWBSI funds approximately 125 projects each year, providing support for graduate student and postdoctoral research; thus the USWBSI also plays a vital role in training the next generation of plant pathologists and plant breeders.

UG99 STEM RUST INITIATIVE

In 1999-2000, a new, highly virulent form of wheat stem rust disease, named Ug99, was found in Uganda. Because the Ug99 mutant can destroy most of the wheat varieties planted worldwide, the international wheat research community was called to action. ARS led the U.S. response effort and devised the Ug99 Action Plan (www.ars.usda.gov/ug99/actionplan.pdf). Subsequently, ARS researchers released the first U.S. wheat with Ug99 resistance ('Appalachian White'), and identified specific Sr genes (stem rust resistance genes) in U.S. wheat varieties and elite germplasm. ARS established the International Winter Wheat Stem Rust Resistance Nursery with CIMMYT in 2008, where 100 new stem rust-resistant wheat lines are distributed each year to breeders in 23 countries around the world. ARS led the field evaluation of all U.S. wheat and barley varieties and germplasm in East Africa, beginning in 2005 when 4,000 U.S. wheat and barley varieties and germplasm per year from 28 University breeding programs, 12 private companies, and 8 ARS locations were evaluated. ARS organizes the vernalization, planting, evaluation, and harvesting of the 4,000 U.S. lines with the Kenyan Agricultural Research Institute (KARI) and the Ethiopian Institute of Agricultural Research (EIAR).

In addition, ARS scientists led in the development of wheat and barley with resistance to Ug99. The identification of three stem rust resistance genes showed conclusively that intrinsic resistance to Ug99 was present in many U.S. winter wheats. Beginning in 2009, ARS scientists trained 120 scientists from developing countries in Njoro, Kenya, through the Borlaug Global Rust Initiative at a 2-week course in classroom, laboratory, and field methodologies in the standardization of wheat germplasm evaluation to rust diseases. The strategic goals of this initiative span the programmatic range of establishing a national communications network, demonstrating effective disease management systems, understanding host-pathogen interactions, developing enhanced germplasm, improving knowledge of pathogen biology, and developing decision models to guide the implementation of control measure.

NATIONAL SCLEROTINIA INITIATIVE

The National Sclerotinia Initiative is an integrated and collaborative, cross-commodity approach for developing diagnostic technologies, disease management systems, genomic resources, and crop germplasm with durable resistance to *Sclerotinia sclerotiorum*, also known as white mold. The Initiative, coordinated by ARS, was implemented to combat Sclerotinia diseases in seven crops (canola, dry bean, pea, lentil, chickpea, soybean, and sunflower). Supported by 73 ARS projects and specific cooperative agreements with 17 universities and agricultural experiment stations in 13 U.S. states and one station in Canada, the Sclerotinia Initiative has resulted in 21 germplasm releases (5 crops) with improved tolerance to white mold; a method for assessing regional variation in common bean to isolates of the pathogen and a database of the uniform trials (www.css.msu.edu/bic/); fast tracking Sclerotinia resistance in hybrid sunflower; and a grower-

oriented forecasting system, consisting of a general risk map and a risk calculator to assist producers with managing Sclerotinia in canola (www.ag.ndsu.edu/sclerotinia).

SOYBEAN RUST INITIATIVE

The Soybean Rust Initiative was implemented in response to the 2009 introduction of Asian soybean rust into the United States. USDA facilitated the development of a Federal/State/industry coordinated framework for protecting U.S. soybean production against the potentially devastating invasive pathogen. The cooperating USDA agencies included NIFA, ARS, and APHIS. The integrated research program supported by this initiative covered all aspects of the pathogen and the host, as well as host-pathogen interactions to ultimately release resistant cultivars that have been incorporated into breeding programs. The Soybean Rust Initiative discovered the soybean rust SSR markers at *Rpp1* locus, and developed and released germplasm and commercial cultivars of soybean cultivars with resistance or tolerance to soybean rust.

NATIONAL PLANT DISEASE RECOVERY SYSTEM

The National Plant Disease Recovery System (NPDRS) is authorized by Homeland Security Presidential Directive Number 9 (HSPD-9), issued in February of 2004. The purpose of the NPDRS is to ensure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks such that a reasonable level of crop production is maintained in the United States. In addition to the development of recovery plans for individual plant diseases, NPDRS also supports research on emerging plant disease threats. NPDRS funds provide the means to acquire critical data for initial assessment of emerging disease problems or for development of emergency mitigation measures while more sustainable management strategies are in development. NPDRS-funded research has had strong impact in identification of races of the soybean rust pathogen; development of lures for the insect vector of the Laurel wilt fungus; rescue and quarantine of avocado germplasm from areas not yet reported to have Laurel wilt; host range information on the Powell Butte nematode; and screening of U.S. wheat and barley germplasm in Kenya for resistance to newly emerging, highly-virulent races of the stem rust pathogen. Additional information regarding the NPDRS can be found at www.ars.usda.gov/research/npdrs.

FLORICULTURE AND NURSERY RESEARCH INITIATIVE

Disease management and resistance development is an integral part of the Floriculture and Nursery Research Initiative (FNRI), which was established in 1998 to address the research priorities of the floriculture and nursery industries. Research projects, lead by both ARS and university scientists, are initiated after extensive consultation with industry to complement research supported by the nursery industry's research funding arm, the Horticultural Research Institute (HRI), and by the floriculture industry's American Floral Endowment (AFE) and Gloeckner Foundation. Recent research is targeted at effective pathogen and pest control; ornamental genetic improvement; the establishment and expansion of ornamental germplasm collections of herbaceous and woody plants; improved, sustainable plant production methods; new ways to reduce the introduction of invasive plants; water use efficiency; new planting substrates; carbon sequestration; and adaptation to climate change for a sustainable future.

Under the FNRI, specific accomplishments relating to NP 303 include the development and release of new herbaceous and woody ornamentals with improved disease and insect pest resistance; integrated systems for management of critical diseases for these crops; determination of mechanisms of disease development for pathogens caused by oomycetes and select agents, such as *Ralstonia solanacearum*. The etiology and epidemiology of important viruses affecting the ornamental trade has been addressed as well, including suppression methods of viral vectors for disease management, cultural means of disease control, and improved diagnostics.

Funds from the FNRI are also used to host a triennial research meeting of Initiative researchers. The most recent meeting was held in 2009, where approximately 80 ARS and university researchers presented their research progress and conferred with floral and nursery industry leaders for input on priority research direction. (www.ars.usda.gov/docs.htm?docid=19658)

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COMPONENT 4: Biological and Cultural Strategies for Sustainable Disease Management

Most modern agricultural systems require intensive input of energy, synthetic pesticides, and fertilizers. Devising effective plant disease management strategies that are more sustainable is needed to improve economic viability, environmental security, and improved worker safety, and to reduce human exposure to inappropriate use of pesticides. Alternative control measures in an integrated management system can reduce the number of pesticide applications, and rotation of pesticides with alternatives can contribute to managing the occurrence of pathogen resistance to pesticides. In addition, the loss of some pesticides from the market, particularly methyl bromide for preplant soil fumigation, has created an urgent need for alternatives. While alternatives to methyl bromide are actively investigated under NP 308 (Alternatives to Methyl Bromide), the research in Component 4 is closely aligned with research in that national program.

ARS scientists working within this component have made substantial advances in developing and improving sustainable disease management tools, in elucidating pathogen-plant-antagonist-host interactions, and in determining the traits and genes important in biocontrol and plant colonization.

PROBLEM STATEMENT 4A: *Biological and Cultural Control Technologies*

Crop rotation, timing of planting, incorporation of organic matter into soil, and raised beds, are among the oldest cultural techniques available for management of plant diseases and are important components of disease management today. ARS scientists have developed or improved methods for nematode management and soil disinfestation, optimized fungicide timing to reduce the number of sprays, and used molecular genetics and comparative genomics of biocontrol bacteria to identify genes associated with biocontrol and root colonization. Identification of these genes may lead to improvements in use of biocontrol agents, methods to screen for new agents, or even plant varieties developed to foster communities of biocontrol agents. Newly developed molecular techniques facilitate a greater understanding of the mechanisms underlying cultural techniques, and thus offer the possibility of optimizing use of cultural practices.

ARS scientists and their collaborators discovered and developed many of the approximately 30 microbes EPA has registered for biocontrol of plant diseases. Many biocontrol agents have specialized uses, such as protection of apple and pear blossoms from infection by the fire blight pathogen or coating fruit with yeasts to prevent postharvest decay, while others have more general use, such as those against soilborne pathogens.

The Action Plan identified three anticipated Outputs that were expected from ARS research addressing the needs expressed in Problem Statement 4A. Accomplishments that illustrate those products for this problem statement follow the list of anticipated Outputs.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. Discovery and description of ecologically based methods of disease control.
2. Characterization of the genomes and basic biology of selected biocontrol agents.
3. Initial development and testing of new sustainable cropping and management systems that promote plant disease control.
4. Novel physical and chemical treatments for reducing plant disease.

PROBLEM STATEMENT 4A: SELECTED ACCOMPLISHMENTS

Output 1: ***Discovery and description of ecologically based methods of disease control.***

Anaerobic soil disinfestation. Many types of plant pathogens inhabit soil. Populations of these pathogens must be eliminated or reduced below an economic threshold before planting for crop production to be profitable. Previously, this was accomplished primarily with methyl bromide and other soil fumigants. ARS scientists in Fort Pierce, Florida, in cooperation with university colleagues, developed a soil-disinfestation technique using a combination of composted chicken broiler litter and a carbon source, such as molasses, along with soil saturation and heating to create an anaerobic condition that induces control of soilborne pests and pathogens. When soil was amended with both litter and molasses, the effect on anaerobicity was greater than that of either broiler litter or molasses alone. Reduction of inoculum of the pathogen *Phytophthora capsici* was equal to that of methyl bromide except the untreated check. There was an indication that numbers of non-pathogenic, beneficial nematodes increased in some treatments. Ongoing research combines this method with plastic films to improve weed control for sustainable methods for soil disinfestation in Florida strawberry production.

Rosskopf, E. 2011. Field Evaluation of non-fumigant pest control for Florida strawberry production. 2011 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. 45.

Tomato ringspot management. *Tomato ringspot virus,* which is vectored by the dagger nematode, attacks a wide variety of crops, including raspberry. ARS scientists in Corvallis, Oregon, and collaborators demonstrated that rotation of crops was as effective as soil fumigation in reducing the reoccurrence of *Tomato ringspot virus* when planting raspberry on sites with a history of the virus. In addition, modifying cultivation and the use of ground cover plants significantly limited the spread of the virus in established plantings. These methods are cost-effective and sustainable for growers.

Pinkerton, J.N. and Martin, R.R. 2005. Management of tomato ringspot virus in red raspberry with crop rotation. International Journal of Fruit Science 5:55-67

Walters, T.W., Pinkerton, J.M., Riga, E., Zasada, I.A., Particka, M., Yoshida, H., Ishida, H. 2009. Managing plant-parasitic nematodes in established red raspberry fields. HortTechnology 19:762-768.

Output 2: ***Characterization of genomes and basic biology of selected biocontrol agents.***

Genomics-enabled identification of new metabolites contributing to biological control. Genomic sequences of biocontrol agents provide information about traits associated with biocontrol, which can be used to screen for new biocontrol agents. Working with collaborators, ARS scientists in Corvallis, Oregon, developed and employed genomic mining approaches to identify novel traits from the genome of the biological control organism *Pseudomonas fluorescens* strain Pf-5. Metabolites identified include orfamide A, the founding member of a novel class of cyclic lipopeptides, and derivatives of rhizoxin that exhibit antifungal properties. After discovering a gene encoding an insect toxin (FitD) in the genome of Pf-5, the scientists demonstrated that Pf-5 exhibits insect toxicity when injected into tobacco hornworms (*Manduca sexta*) or when ingested by larvae of the fruit fly *Drosophila melanogaster*. Together, these studies demonstrate the value of genomics in the discovery of new traits with antifungal and insecticidal activities. Recently, the research team sequenced the genomes of seven well-characterized biological control strains of *Pseudomonas* spp. Through comparative genomic analyses, the scientists identified approximately 2,800 genes that are shared by all strains and 300 to 900 genes that are unique to each strain. In the unique regions of each genome, the scientists discovered new gene clusters with potential roles in biological control, including those encoding insect toxins and the biosynthesis of new secondary metabolites. These gene clusters provide avenues for the future discovery of novel natural products, including those contributing to biological control of plant disease. The researchers are also discovering the genetic basis for biocontrol and plan to exploit the knowledge to improve plant health.

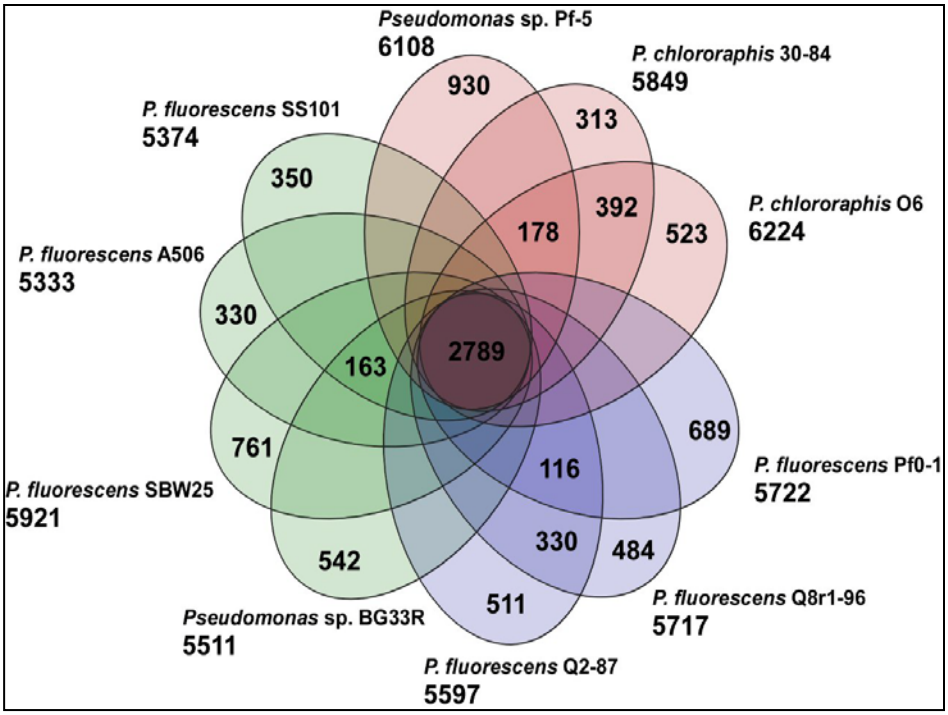

Figure 8: Genomic diversity of biological strains of *Pseudomonas* spp. is illustrated in this graphic. The total number of protein-coding genes in each genome is listed below the strain name. The core genome (center) is composed of 2,789 genes present in all strains, representing approximately one half of each strain's genome. Each strain has ~300 to ~900 unique genes and these genes are being evaluated to identify new traits contributing to the distinctive biological control properties of each strain. *Graphic by Joyce Loper/ARS.*

Olcott, M. H., Henkels, M. D., Rosen, K. L., L. Walker, F., Sneh, B., Loper, J. E., and Taylor, B. J. 2010. Lethality and developmental delay in *Drosophila melanogaster* larvae after ingestion of selected *Pseudomonas fluorescens* strains. Plos ONE 5:e12504.

Gross, H., and Loper, J. E. 2009. Genomics of secondary metabolism in *Pseudomonas* spp. Natural Product Reports 26:1408-46.

Loper, J. E., Henkels, M. D., Shaffer, B. T., Valeriote, F. A., and Gross, H. 2008. Isolation and identification of rhizoxin analogs from *Pseudomonas fluorescens* Pf-5 by using a genomic mining strategy. Applied & Environmental Microbiology 74:3085-3093.

Pasteuria penetrans for management of Meloidogyne arenaria. The bacterium *Pasteuria penetrans* parasitizes the root-knot nematode *M eloidogyne arenaria*, which attacks vegetables and peanuts. ARS scientists in Tifton, Georgia, discovered that strains of *P. penetrans* are extremely host specific and isolates of the bacterium differentially adhere to populations within a Meloidogyne species. While *P. penetrans* has been successful in field tests, there has also been an indication that *M. arenaria* was able to build up resistance to the bacterium. The intellectual property rights to ARS strains of *P. penetrans* have been acquired by Syngenta. ARS continues collaboration with the company in developing this biocontrol agent as a sustainable management tool for *M. arenaria*.

Timper, P. 2009. Population dynamics of *Meloidogyne arenaria* and *Pasteuria penetrans* in a long-term crop rotation study. Journal of Nematology 41:291-299.

Output 3: ***Initial development and testing of new sustainable cropping and management systems that promote plant disease control.***

Solarization in cut-flower production. The cut-flower industry relied greatly on the use of methyl bromide for preplant soil fumigation to manage soilborne pathogens. ARS scientists in Fort Pierce, Florida, with their industry and university collaborators developed systems for management of fungal pathogens and root knot nematode in soil using solarization alone, and in combination with steam. Based upon favorable grower assessment of performance and resulting yield and quality of cut-flower production, the acreage treated with soil solarization was expanded for the 2011 production season.

Kokalis-Burelle, N., Rosskopf, E.N., Hartman, R.D. 2010. Evaluation of soil treatments for control of *Meloidogyne arenaria* in Caladium tubers (*Caladium x hortulanum*) and nematode susceptibility of selected cultivars. Nematropica 40:177-189.

McSorley, R., Wang, K., Rosskopf, E.N., Burelle, N.K., Hanspetersen, H., Gill, H., Krueger, R. 2009. Nonfumigant alternatives to methyl bromide for production of snapdragon (*Antirrhinum majus*). International Journal of Pest Management 55(4):265-273.

Grafting watermelon to control root-knot nematode. Root-knot nematodes cause extensive damage to root systems of watermelon and many other vegetable crops, resulting in significant yield losses. ARS scientists in Charleston, South Carolina, identified wild watermelon lines resistant to root-knot nematodes, and that performed well as rootstocks, producing high yields from the resulting grafted watermelon plants. These rootstocks are being used as alternatives to soil fumigation with pesticides such as methyl bromide for managing root-knot nematodes in watermelon fields. These rootstocks have been made available to seed companies interested in developing rootstock varieties for grafted watermelon.

Thies, J.A., Ariss, J., Hassell, R., Kousik, C.S., Olsen, S., Levi, A. 2010. Grafting for managing southern root-knot nematode, *Meloidogyne incognita*, in watermelon. Plant Disease 94:1195-1199.

Fungicide timing. Sugar beet growers traditionally relied on the fungicide azoxystrobin to manage Rhizoctonia root and crown rot. The fungicides polyoxin-d and flutolanil are used to control *Rhizoctonia solani* in several pathosystems, but information about the efficacy of these fungicides to control Rhizoctonia on sugar beet was limited. ARS researchers in Fargo, North Dakota, found that all three fungicides were able to lower disease severity by nearly half relative to the water control treatment. They also determined the temperature and moisture conditions most favorable to disease development so that fungicides could be targeted to the time disease is expected. This information has saved sugarbeet growers money and avoids unnecessary pesticides in the environment.

Bolton, M.D., Panella, L.W., Campbell, L.G., Khan, M.F. 2010. Temperature, moisture, and fungicide effects in managing Rhizoctonia root and crown rot of sugar beet. Phytopathology 100(7):689-697.

Output 4: ***Novel physical and chemical treatments for reducing plant disease.***

Novel seed treatment. Treating seed to kill seedborne plant pathogens can reduce seed germination and establishment. ARS scientists in Frederick, Maryland, and collaborators investigated novel physical and chemical treatments for potential in ridding watermelon seeds of the bacterial fruit blotch pathogen *Acidovorax avenae* subsp. *citrulli*. They discovered that using acidic electrolyzed water for 30 minutes kills the bacterial fruit blotch pathogen on watermelon seeds without reducing seed germination or seedling establishment. These findings provide a new method to ensure that seeds are pathogen-free prior to planting, a critical means of excluding transmission of this disease in watermelon production.

Feng, J., Li, J., Randhawa, P., Bonde, M.R., Schaad, N.W. 2009. Evaluation of several seed treatments for eradication of *Acidovorax avenae* subsp. *citrulli* from watermelon seed. Canadian Journal of Plant Pathology 31:180-185

Systemic acquired resistance to mitigate Asian soybean rust. Rusts diseases are usually managed with resistant cultivars, but when new races of rust develop or exotic rusts are introduced, alternative methods are needed until resistance can be incorporated into new cultivars. Since Asian soybean rust (*Phakopsora pachyrhizi*) was first detected in the United States in November 2004, the judicious use of fungicides has been recommended for management of this disease. ARS scientists in Urbana, Illinois, and their collaborators investigated saccharin – which has been reported to induce resistance to diseases in other plant systems – as a lower cost, more environmentally benign alternative to fungicides. Saccharin induced systemic acquired resistance and reduced the severity of Asian soybean rust; with a root drench being more effective than a foliar spray treatment. Systemic protection against rust infection was still apparent 15 days after application of saccharin as a root drench. In contrast, the foliar treatment did not increase systemic protection until 15 days after treatment. When systemic protection was induced by the application of saccharin in either manner, there was no significant reduction of plant growth except when saccharin was applied as a drench 15 days after treatment at the early flowering (R1) stage of soybean development. Use of these findings as part of an overall management strategy for rust fungi is being explored.

Srivastava, P., George, S., Marois, J.J., Wright, D.L., Walker, D.R. 2011. Saccharin-induced systemic acquired resistance against rust (*Phakopsora pachyrhizi*) infection in soybean: Effects on growth and development. Crop Protection 30(6):726-732.

PROBLEM STATEMENT 4B: *Pathogen, Plant, and Antagonist Interactions*

Knowledge of the mechanisms of biocontrol and of interactions among pathogens, antagonists, plants, and their environment can help increase the reliability of current controls and suggest new approaches for biocontrol. ARS accomplishments in Problem Statement 4B range from detailed information on interactions in a model biocontrol system to enhancing biocontrol in field situations through new knowledge and novel technologies.

The Action Plan identified three anticipated Outputs that were expected from ARS research addressing the needs expressed in Problem Statement 4B. Accomplishments that illustrate those products for this problem statement follow the list of anticipated Outputs.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. Improved knowledge of the mechanisms by which biological, chemical, and physical treatments suppress disease and the interactions between the plant, microbe, and environment.
2. Enhanced levels of disease control for selected pathosystems via application of the knowledge of system-specific interactions occurring between pathogen, plant, and antagonist.

3. Tools and methods for evaluating and describing system-specific interactions and the success of ecologically based disease control practices.

PROBLEM STATEMENT 4B: SELECTED ACCOMPLISHMENTS

Output 1: ***Improved knowledge of the mechanisms by which biological, chemical, and physical treatments suppress disease and the interactions between the plant, microbe, and environment.***

Biocontrol mechanisms of Pseudomonas fluorescens Q8r1-96. The biocontrol bacterium *Pseudomonas fluorescens* Q8r1-96 represents a group of root-inhabiting beneficial bacteria responsible for the natural biocontrol of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*. ARS scientists in Pullman, Washington, and collaborators analyzed the genome of Q8r1-96 and identified genes that might account for the ability of this strain to aggressively colonize wheat roots. In Q8r1-96, and in 29 of 30 related strains, a gene cluster encoding a type III protein secretion system (T3SS) similar to that in the plant pathogen *Pseudomonas syringae* was identified. The T3SS acts as a molecular syringe that can inject bacterial proteins called effectors into cells of their host, modifying host cell behavior and suppressing plant defense responses. The Q8r1-96 genome encodes three effector proteins that are secreted in culture and injected into plant cells. The T3SS of Q8r1-96 is expressed during wheat root colonization and mutants lacking a functional T3SS were mildly altered in root colonizing ability. The scientists determined that defense/stress genes of roots of some wheat cultivars were induced more quickly and showed a more comprehensive pattern of host defense response induction than those of other wheat cultivars, demonstrating that at least some aspects of root gene expression underlying biocontrol is cultivar dependent. A more thorough understanding of plant immunity and root colonization is expected to lead to improved biocontrol.

Kwak, Y., Han, S., Thomashow, L.S., Topham, J., Paulitz, T.C., Kim, D., Weller, D.M. 2011. *Saccharomyces cerevisiae* genome-wide mutant screen for sensitivity to 2,4-diacetylphloroglucinol, a biocontrol antibiotic produced by *Pseudomonas fluorescens*. Applied and Environmental Microbiology 77:1770-1776.

Mavrodi, D.V., Joe, A., Mavrodi, O., Hassan, K.A., Weller, D.M., Paulsen, I.T., Loper, J.E., Alfano, J.R., Thomashow, L.S. 2011. Structural and functional analysis of the type III secretion system from *Pseudomonas fluorescens* Q8r1-96. Journal of Bacteriology 193:177-189.

Okubara, P. A., Call, D. R., Kwak, Y.-S., and Skinner, D. Z. 2010. Induction of defense gene homologues in wheat roots during interactions with *Pseudomonas fluorescens*. Biological Control 55:118-125.

Okubara, P. A., and Bonsall, R. F. 2008. Accumulation of *Pseudomonas*-derived 2,4-diacetylphloroglucinol on wheat seedling roots is influenced by host cultivar. Biological Control 46:322-331.

Sexual reproduction responsible for variation in aflatoxin production by Aspergillus. Aflatoxins are produced by the fungus *Aspergillus flavus* and are the most carcinogenic naturally occurring substances known, but not all strains of *A. flavus* produce aflatoxins. In 2006, ARS sceinitsts in Dawson, Georgia, transferred to industry an atoxigenic strain of *A. flavus* that reduces aflatoxin contamination in peanut by 70-90 percent and is now commercially available as Afla-guard®. These scientists and their university collaborators have now uncovered information that improved understanding of the origins of genetic variation in aflatoxin-producing Aspergillus. The major aflatoxin-producing fungi, *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, were previously considered to be strictly asexual in reproduction. This research identified mating-type genes and strains of opposite mating type were crossed, resulting in the formation of the sexual stage in all three species. Approximately 3,500 progeny strains from sexual crosses were generated. Examination of progeny showed that meiosis results in genetic recombination of the aflatoxin gene cluster due to independent assortment of chromosomes and crossing over within the gene cluster. This confirmed that sexual reproduction is responsible for the genetic variation in aflatoxin production by Aspergillus in crops. The discovery of sexuality redefines all that was previously known about the biology of aflatoxin.

Abbas, H. K., Zabkotowicz, R. M. 2008. Biocontrol of aflatoxin of corn by inoculation with non-aflatoxigenic *Aspergillus flavus* isolates. U. S. Patent No. 7361499.

Horn, Bruce W., Moore, Geromy G., Carbone, Ignazio. 2009. Sexual reproduction in *Aspergillus flavus.* Mycologia 101:423-429.

Horn, Bruce W., Ramirez-Prado, Jorge H., Carbone, Ignazio. 2009. Sexual reproduction and recombination in the aflatoxin-producing fungus *Aspergillus parasiticus*. Fungal Genetics and Biology 46:169–175.

Output 2: *Enhanced levels of disease control for selected pathosystems via application of the knowledge of system-specific interactions occurring between pathogen, plant, and antagonist.*

Biocontrol mechanisms of brassicaceous seedmeal with Pseudomonas fluorescens SS 101. For nearly 50 years, replant diseases were managed by fumigating soil with methyl bromide prior to replant. In response to the loss of methyl bromide, ARS scientists in Wenatchee, Washington, developed effective non-fumigant and non-chemical methods for managing apple replant disease, which is incited by a complex of *Pratylenchus penetrans*, *Pythium* spp., *Rhizoctonia solani*, *Cylindrocarpon spp.*, and *Phytophthora* spp. They determined that brassecacous seedmeals were effective managing these pathogens, but control was rootstock dependent. Even on the susceptible apple rootstock M26, seedmeals were as effective as preplant 1, 3-dichloropropene-chloropicrin soil fumigation in terms of disease control, vegetative tree growth, and 5-year fruit yields for Gala apple. The scientists also showed that certain Rhizobacteria, especially *Pseudomonas fluorescens* strain SS 101, use multiple mechanisms to control *Pythium* spp. Autumn or spring applications of the rhizobacteria were shown to be

effective in higher organic matter loam soils, but in light-textured sandy soils extended plant-back periods were needed due to potential phytotoxicity of the combined treatment. The scientists found that these bacteria produce cycliclipopeptides that inhibit plant pathogenic fungi and are partially responsible for biological control of replant disease. Growers for Driscoll Fruit in California are using this method, as well as individual strawberry and raspberry growers.

Mazzola, M., Brown, J. 2010. Efficacy of *Brassicaceous* seed meal formulations for the control of apple replant disease in conventional and organic production systems. Plant Disease. 94:835-842.

Mazzola, M., Brown, J., Zhao, X., Izzo, A., Fazio, G. 2009. Interaction of *Brassicaceous* seed meal and apple rootstock on recovery of *Pythium* spp. and *Pratylenchus penetrans* from roots grown in replant soils. Plant Disease. 93:51-57.

Output 3: ***Tools and methods for evaluating and describing system-specific interactions and the success of ecologically based disease control practices.***

Improved fire blight forecasting. Under conditions of proper sanitation that eliminate infection through inoculum on pruning shears, the fire blight pathogen will only infect apple and pear trees through the base of the flower. Due to the limited time (flowering) and the small area of the plant that needs protection, biocontrol of fire blight reduces the number of fire blight strikes by approximately 40 to 80 percent under commercial conditions. Improvements in disease forecasting can be coupled with the timing of biocontrol (or antibiotic) applications to further refine control. ARS scientists in Wenatchee, Washington, and collaborators discovered critical information on microbial growth and interactions on floral surfaces as related to flower age for the fire blight pathogen and its biocontrol agent. This information was used to improve a model for fire blight risk assessment by more precisely relating hourly and daily high temperatures to the expected size of the pathogen population. The new version is called CougarBlight 2010 and is widely used by growers in the Pacific Northwest.

Smith, T. J., and Pusey, P.L. 2011. CougarBlight 2010, A significant update of the CougarBlight fire blight infection risk model. Acta Horticulturae 896:331-336.

Problem Statement 4C: ***Application of Sustainable Disease Management Tools***

One of the difficulties in using biocontrol to manage plant diseases is the production and formulation of a living microbe. Production and formulation can affect the viability, shelf-life, efficacy, worker safety, and economic feasibility of delivering a living agent to the field. ARS scientists have developed new methods for more efficient production of biocontrol agents, are developing new biocontrol strategies, and have transferred biocontrol to use by growers.

The Action Plan identified three anticipated Outputs that were expected from ARS research addressing the needs expressed in Problem Statement 4C. Accomplishments that illustrate those products for this problem statement follow the list of anticipated Outputs.

ANTICIPATED OUTPUTS IN ACTION PLAN:

1. New production and formulation technologies that enhance the efficacy of biocontrol agents and natural products.
2. Basic and applied information on integrating biological, cultural, and chemical control strategies to reduce plant disease.
3. Biological control and natural products for protecting agriculturally important plants and/or minor crops from diseases.

PROBLEM STATEMENT 4C: SELECTED ACCOMPLISHMENTS

Output 1: ***New production and formulation technologies that enhance the efficacy of biocontrol agents and natural products.***

Biocontrol yeasts for wheat scab management. Fusarium head blight of wheat (FHB) caused by the fungus *Fusarium graminearum* is a serious problem in wheat and barley, not only because of the direct losses, but also because the pathogen produces the mycotoxin deoxynivalenol (DON) that poses a health risk to humans and livestock. Fungicides such as prothioconazole are applied to wheat to manage FHB, but use of these fungicides is restricted by the pesticide label after wheat plants flower. As part of the U.S. Wheat and Barley Scab Initiative (www.scabusa.org/index.php?id=26; see also Component 3), ARS scientists in Peoria, Illinois, discovered strains of yeasts, including *Cryptococcus flavescens*, that could reduce the severity of FHB. To improve the usefulness *C. flavescens* in an integrated management strategy against FHB, the scientists selected natural variants of this yeast resistant to the fungicide prothioconazole. They then developed a new production technique to co-culture these yeast strains, resulting in a product that consistently reduced FHB severity. When this product was produced in 100 liter fermentors and field-tested with collaborators in five states in two successive years, a combination yeast co-culture and fungicide treatment was found to be the most effective of multiple treatments, including fungicide alone, in reducing DON in wheat kernels. Recently, three research agreements were established between ARS and industry to evaluate commercializing these yeasts and this technology.

Dunlap, C.A., Schisler, D.A. 2010. Fluidized-bed drying and storage stability of *Cryptococcus flavescens* OH 182.9, a biocontrol agent of Fusarium head blight. Biocontrol Science and Technology 20(5):465-474.

Slininger, P.J., Dunlap, C.A., Schisler, D.A. 2010. Polysaccharide production benefits dry storage survival of the biocontrol agent *Pseudomonas fluorescens* S11:P:12 effective against several maladies of stored potatoes. Biocontrol Science and Technology. Biocontrol Science and Technology 20(3):227-244.

Output 2: ***Basic and applied information on integrating biological, cultural, and chemical control strategies to reduce plant disease.***

Biocontrol of fire blight. Combining biocontrol agents can result in synergistic, additive, negative, or no effect on biocontrol efficacy and consistency. ARS scientists in Corvallis, Oregon, found that when two commercially available bacteria for fire blight management were used together, they were mechanistically incompatible. *Pseudomonas fluorescens* A506 (BlightBan A506; NuFarm) protects flower surfaces through competitive exclusion, while production of an antibiotic is key to fire blight control by *Pantoea vagans* C9-1 (BlightBan C9-1; NuFarm). By comparing the biocontrol ability of *P. vagans* C9-1 plus *P. fluorescens* A506, with that of a mutant of *P. vagans* C9-1 that could not produce the antibiotic, plus *P. fluorescens* A506 in field tests, the scientists determined that the antibiotic produced by *P. vagans* C9-1was interfering with *P. fluorescens* A506. ARS scientists also discovered a new biocontrol agent, *Pantoea agglomerans* E325, for fire blight of apple and pear (U.S. Patent No. 5,919,446) that has been made available commercially and is now used in fire blight management.

Pusey, P.L., Stockwell, V.O., Rudell Jr., D.R. 2008. Antibiosis and acidification by *Panoea agglomerans* strain E325 may contribute to suppression of *Erwinia amylovora*. Phytopathology 98:1136-1143.

Stockwell, V., Johnson, K.J., Sugar, D., Loper, J.E. 2011. Mechanistically compatible mixtures of bacterial antagonists improve biological control of fire blight of pear. Phytopathology 101(1):113-123.

Wang, D., Korban, S.S., Pusey, P.L., Zhao, Y. 2011. Characterization of the RcsC sensor kinase from *Erwinia amylovora* and other enterobacteria. Phytopathology 101:710-716.

Output 3: ***Biological control and natural products for protecting agriculturally important plants and/or minor crops from diseases.***

Aflatoxin management in peanut and corn. Peanuts are commonly invaded by *Aspergillus flavus* and *A. parasiticus,* soil-inhabiting molds that contaminate peanuts by producing carcinogenic aflatoxins. ARS scientists in Dawson, Georgia and collaborators discovered, developed, and transferred to industry native, nontoxigenic strains of Aspergillus that reduce aflatoxin contamination of peanuts in the field by 77 to 98 percent by competitive exclusion. The resulting commercial product, known as Afla-guard®, has been approved by the U.S. Environmental Protection Agency (EPA) for use on peanuts and is being used commercially for that crop.

The scientists have now extended use of Afla-guard® to corn. They applied Afla-guard® at rates of 10 or 20 pounds per acre to commercial fields of corn in 2007 and 2008 in Texas. Results showed that each treatment rate produced significant reductions in aflatoxin in both years with

no difference between the two rates, and aflatoxin was reduced in corn by 85 to 88 percent. Based on these data, Afla-guard® has been recently registered by the EPA for use on corn.

Biocontrol treatments discovered and developed by ARS and collaborators are being used in Africa to mitigate aflatoxin contamination. USDA recently pledged resources, including biocontrols, to combat aflatoxin contamination in Africa under the Feed the Future initiative. A patent is pending for a new ARS-developed formulation of Aspergillus biocontrol agents encapsulated in a plastic that safely biodegrades in the environment.

Dorner, J.W. 2010. Efficacy of a biopesticide for control of aflatoxins in corn. Journal of Food Protection. 73:495-499.

Horn, B.W., Dorner, J.W. 2009. Effect of nontoxigenic *Aspergillus flavus* and *A. parasiticus* on aflatoxin contamination of wounded peanut seeds inoculated with agricultural soil containing natural fungal populations. Biocontrol Science and Technology 19:249-262.

Biocontrol of cacao diseases. While cacao is not produced in the United States, the U.S. chocolate industry is the single largest market for milk and sugar, and a large market for nuts of all kinds. Cacao grows and flowers throughout the year, and thus leaf, flower, and pod tissue susceptible to pathogen infection are constantly available. For worker safety and to protect the ecologically-sensitive areas where cacao is produced as an understory tree, producers have sought alternatives to synthetic pesticides and copper sprays. Trichoderma strains and new species discovered in Brazil by ARS scientists in Beltsville, Maryland, and their Brazilian collaborators are being used by growers in Brazil and elsewhere to mitigate diseases of cacao, including black pod (*Phytopthora* spp.), frosty pod (*Moniliophthora rorei*), and witches' broom (*Moniliophthora (=Crinipellis) perniciosa*). Recently, these scientists discovered that *Trichoderma hamatum* isolate DIS 219B grows endophytically in cacao, promoting growth and delaying the onset of drought stress. The scientists are extending the use of these Trichoderma strains from the tropics to other crops. They recently discovered that these strains also induce resistance to *Phytophthora capsici* in hot pepper. The information was transferred to the agricultural community in Brazil by the Brazilian collaborators and these biocontrol strains are used in cacao production in Brazil.

Bae, H., Roberts, D.P., Lim, H.S., Strem, M.D., Park, S., Ryu, C., Melnick, R., Bailey, B.A. 2011. Endophytic Trichoderma isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. Molecular Plant-Microbe Interactions 24:336-351.

Bailey, B.A., Strem, M.D., Wood, D.F. 2009. Trichoderma species form endophytic associations within *Theobroma cacao* trichomes. Mycological Research 113:1365-1376.

Bae, H., Kim, S., Sicher, Jr., R.C., Kim, M.S., Strem, M.D., Bailey, B.A., Melnick, R. 2009. The beneficial endophyte, *Trichoderma hamatum*, isolate DIS 219B promotes growth and delays the onset of the drought response in *Theobroma cacao*. Journal of Experimental Botany 60:3279-3295.