Recovery Plan

for

Ralstonia solanacearum Race 3 Biovar 2

Causing Brown Rot of Potato, Bacterial Wilt of Tomato, and Southern Wilt of Geranium

October 11, 2006

Contents Page

This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery. .

Executive Summary

Bacterial wilt is an important disease of many crops and is caused by various subgroups of the bacterial pathogen *Ralstonia solanacearum*. *R. solanacearum* was subdivided into 5 races based loosely on host range, and into 5 biovars based on ability to produce acid from a panel of carbohydrates. Race 1 of the pathogen is established in the southern United States, where it causes bacterial wilt on tomato and some other crops. Bacterial wilt caused by race 1 has not spread throughout the United States because the seed potato system is isolated from production system and it is thought that strains of race 1 cannot survive in colder areas outside of the southern United States. In contrast, *R. solanacearum* race 3 biovar 2 (Rsr3b2) can survive in more temperate climates, making it a serious threat to American agriculture, especially to potato production.

Although Rsr3b2 is widely distributed in Asia, Africa, and South and Central America, and is found in some soils and waterways in Europe, it is not known to be established in North America. A pathway for Rsr3b2 into the US has been through the introduction of geranium cuttings produced in the highland tropics of Africa and Central America. Geraniums imported into the US on several occasions in 1999 and 2000 were positive for Rsr3b2. In February 2003, the bacterium was again identified in geraniums imported from a facility in Kenya. The 2003 introduction resulted in the organism being detected in 127 individual greenhouses in 27 states. The organism was detected again in December of 2003 and January of 2004 in geranium cuttings that originated in Guatemala. Rsr3b2 is listed in the US as a "Select Agent", a designation under the Agriculture Bioterrorism Protection Act of 2002, requiring entities, such as private, State, and Federal research laboratories or universities; that possess, use, or transfer select agents products register these agents with USDA, APHIS Plant Protection and Quarantine.

Rsr3b2 causes brown rot, or bacterial wilt, of potatoes. Bacterial wilt of potato has been estimated to affect 3.75 million acres in approximately 80 countries with global damage estimates currently exceeding $950 million per year. It is adapted to cooler temperatures and could be particularly damaging to potato production regions of the US. While race 1 causes losses to tomato crops in the southern US, the economic impact of potato brown rot caused by Rsr3b2 is unknown in temperate climates such as those where potatoes are grown in the United States.

Protection from losses to Rsr3b2 in the US will be provided mainly by quarantines, required off-shore production standards, early detection, and eradication by host destruction. The management of bacterial wilt caused by Rsr3b2 is difficult, because of its wide host range, the latency of the pathogen in potato tubers and lack of effective control measures on potato. Soil fumigation with vapam, methyl bromide, or chloropicrin is of limited efficacy. A plant-derived volatile compound thymol, not commercialized for agricultural use yet, was found to effectively reduce bacterial wilt on tomato caused by *R. solanacearum* race 1 when used for pre-plant soil fumigation. Only a few acceptable tolerant tomato cultivars are available that provide moderate levels of disease control and their efficacy is limited to different regions. Host resistance when combined with Actigard or thymol is providing satisfactory management of the disease caused by *R. solanacearum* race 1. However, none of these tactics are tested for management of brown rot on potato. Development of effective disease management strategies and improvement of detection and monitoring tools are key components of this recovery strategy. Other research with high priority for effectively mitigating Rsr3b2 was summarized in this report.

Ralstonia solanacearum Race 3 Biovar 2 Causing Brown Rot of Potato, Bacterial Wilt of Tomato, and Southern Wilt of Geranium

Contributor authors: Timur M. Momol, Pingsheng Ji, Jeffery B. Jones of University of Florida; Caitilyn Allen of University of Wisconsin; David J. Norman, Carrie Harmon of University of Florida; Sally A. Miller of Ohio State University; Tim Schubert of Florida Department of Agricultural and Consumer Services; Dave Bell of USDA-RMA; Joel P. Floyd, David Kaplan, and Russ Bulluck of USDA-APHIS; Kent Smith of USDA-ARS, Kitty Cardwell of USDA-CSREES.

Reviewers: Dean W. Gabriel of University of Florida; Timothy P. Denny of University of Georgia; Karen K. Rane of Purdue University, review by the American Phytopathological Society planned for 2007

I. Introduction

Bacterial wilt is an important disease of many crops and is caused by various subgroups of the bacterium *Ralstonia solanacearum*. Historically, *R. solanacearum* was subdivided into five races based loosely on host range, and into five biovars based on ability to produce acid from a panel of carbohydrates. There is no general correlation between races and biovars, however biovar 2 strains are almost always race 3 (and vice versa). The five races of *R. solanacearum* have different host ranges and geographic distributions. Race 1 is a poorly-defined group with a very wide host range and is endemic to the southern United States. Race 2 principally attacks bananas, and is found mainly in Central America and Southeast Asia. Race 3 is distributed worldwide and has primarily been associated with potato. Race 4 affects ginger in much of Asia and Hawaii, and race 5 affects mulberries in China. Recently a more phylogenetically meaningful system has classified *R. solanacearum* into four phylotypes roughly corresponding to geographic origin (Fegan and Prior, 2005). The origin of *R. solanacearum* is not clear, but Hayward (1991) suggests it predates the geological separation of the continents as the bacterium has been found in virgin jungle in South America and Indonesia. However, Race 3 biovar 2 strains are believed to originate in the Andean highlands and are widely distributed in tropical regions throughout the world and some temperate regions such as Europe.

Several Race 1 groups of the pathogen are established in the southern United States, where they cause bacterial wilt of tomato and other crops. This race has not spread throughout the United States, possibly because the pathogen cannot survive in cold soils and/or soils with lower water holding capacity. In contrast, a near-clonal subgroup of *R. solanacearum* known as race 3 biovar 2 (Rsr3b2) can survive in more temperate climates including highland tropics, UK, and the Netherlands. Race 3 infects and kills solanaceous plants (e.g. tomato, potato, eggplant, nightshades) as well as geraniums. The host range of this group is not as wide as race 1. It is a particular concern to the potato industry of the United States and could extend the geographic range of *R. solanacearum* in the United States for other crops (i.e., tomato).

The 8X draft of UW551 (Rsr3b2 strain) genome (Gabriel et al., 2006) was published. This information will facilitate future Race 3 specific molecular diagnostic assay developments.

II. Symptoms

Foliage on potatoes infected with *R. solanacearum* may be stunted, yellowed, and wilted. Early in the infection wilting of leaves may be limited to the top portion of plants. Infected plants may appear to recover at night, but soon wilting becomes irreversible and death of the plants follows. The stems of young plants may collapse and/or have narrow dark streaking present. Vascular discoloration of the stem appears to be grey or brown and bacterial ooze is present. The vascular ring of symptomatic potato tubers will show discoloration and ooze (Fig. 1). Initially the vascular ring appears yellow to light brown, but as the infection progresses the ring will become browner.

On tomatoes, symptoms of bacterial wilt caused by *R. solanacearum* are the same whether disease is caused by race 3 biovar 2 or other groups. Wilt is first seen as a flagging of one or two leaves on plants. The disease develops rapidly and may kill plants as quickly as 4-7 days after appearance of the first wilt symptoms (Fig. 2).

On geranium, the earliest symptom of bacterial wilt may be very subtle, developing as a leaf scorch in sectors and abnormal yellowing of lower leaves. Soon after these initial symptoms appear, geranium plants begin to express wilt symptoms (Fig. 3). Wilting symptoms in geranium caused by *Ralstonia* species are similar to wilting symptoms caused by other pathogens such as *Xanthomonas campestris* pv. *pelargonii*, the causal agent of bacterial blight. Unlike bacterial wilt, bacterial blight can also produce leaf spots.

Fig. 1. Brown rot caused by Rs 3/2 on potato. Photo Credit: The International Potato Center, Lima, Peru.

Fig. 2. Bacterial wilt on tomato caused by *Ralstonia solanacearum* race 1 in Florida.

Fig. 3. Wilting of geranium caused by *R. solanacearum* race 3 biovar 2. Photo Credit: State of Wisconsin Dept. of Trade and Consumer Protection.

Fig. 4. Bacterial streaming in clear water produced by wilted tomato stem cut. Photo Credit: University of Georgia, Plant Pathology Extension.

On all infected plants, bacterial streaming (ooze) may occur upon placing cut main stem material in a test tube with water (Fig. 4). On potato, bacterial ooze may also be present; in later stages this ooze may emerge from the eyes and heel (stolon) end to which soil particles will attach. If the vascular tissue has collapsed, sunken skin lesions will also appear.

Plants infected with *R. solanacearum* do not always show symptoms; such latent infections are common in potatoes and geraniums (Swanson et al., 2005). On the other hand, other plant pathogens (i.e. Fusarium wilt on tomato), root damage, and drought conditions can cause symptoms similar to those of bacterial wilt; therefore, symptoms alone should not be used for diagnosis. For this reason, caution is needed in concluding that a plant with the symptoms described above really has the disease. This is especially true for bacterial wilt caused by Rsr3b2, since similar symptoms in different plants may be caused by race 1 of the pathogen which is endemic in the southern United States. Furthermore, both Rsr3b2 and Rs race 1 will react to the ELISA tests currently available commercially.

III. Spread

Ralstonia solanacearum is disseminated by soil, contaminated irrigation water, surface water, equipment, or personnel as well as infected plant material (including geranium cuttings and seed potatoes) (Janse, 1996). It may be spread by transplanting infected plants, taking cuttings without disinfecting grafting knives between plants, pinching buds of plants, and especially by sub-irrigation or ebb-and-flow irrigation of geraniums. Plants may be infected through tissue damaged from nematodes, handling, and root development. The pathogen does not readily spread from plant-to-plant through the splashing of water, casual contact, or aerially. Spread can

be controlled in greenhouses by the application of sound sanitation practices and discontinued use of ebb-and-flow irrigation.

A mechanism by which Rsr3b2 has entered the US has been through the introduction of latently infected geranium cuttings produced in greenhouses located outside the country. Geranium cuttings imported into the US on several occasions in 1999 and 2000 were positive for *R. solanacearum* Race 3, Biovar 2 (Kim et al., 2002 and 2003, Williamson et al., 2002). During 2001 and 2002 the US had no reported cases of Rsr3b2. In February 2003, the bacterium was again identified in geraniums, this time from a facility in Kenya. The outbreak resulted from a breach in sanitation in Kenya that led to the contamination of 7 stock plants. The 2003 introduction resulted in the organism being detected in 127 individual greenhouses in 27 states. Work was conducted by state and APHIS personnel to contain, destroy, and eradicate any diseased geraniums (Daughtrey, 2003). The organism was detected again in December of 2003 and January of 2004. In addition to state and federal regulatory organizations, the National Plant Diagnostic Network (NPDN) provided early detection, diagnostic and training support to enhance the national surveillance capacities and capabilities. The reintroduction that occurred in December 2003 is not believed to be a result of the contamination from the spring of 2003 because the greenhouse did not receive plants from the Kenya cutting station earlier that year, but from new cuttings that came from Guatemala (O'Hern, 2004).

IV. Monitoring, Detection and Identification

Confirmatory diagnosis of bacterial wilt caused by Rsr3b2 must be made by advanced microbiological and molecular tests. The ultimate authority for confirming a diagnosis of the disease rests with the Plant Protection and Quarantine (PPQ) division of APHIS: http://www.aphis.usda.gov/ppq .

1. Bacterial streaming (Fig. 4): Clouds of bacteria should quickly appear when petioles or stems of the suspected plants are placed in tubes of sterile water. Note that this may not occur when viewing plant material in the early stages of infection. This procedure only indicates that wilt may be caused by a bacterial pathogen.

2. Isolation:

a. SMSA semi-selective medium

The bacteria may be cultured from diseased tissue in a diagnostic laboratory by streaking from the suspensions that flow out from diseased tissue into sterile water onto Petri plates containing SMSA semi-selective medium. The plates should be incubated at 28 °C for 3-5 days. *R. solanacearum* appears as mucoid, whitish colonies after 48-60 hours. The colonies then develop blood red whorls in the center after further incubation. Most colonies that are not *R. solanacearum* will appear red throughout (SMT Project CT97-2179 and new pest response guidelines *Ralstonia solanacearum* USDA). This test is not diagnostic for race or biovar.

It is helpful to observe the microscopic colonies at 24 to 36 hours on SMSA for twitching motility. Relatively few bacteria species will exhibit this distinctive colony morphology and

those that do can be distinguished from *R. solanacearum* once visible colonies develop (Liu et al., 2001).

b. Modified tetrazolium medium (TZC)

TZC medium is preferred for testing strains because growth occurs more rapidly with typical colonies developing within 48-72 hours. The bacteria may be cultured from diseased tissue in a diagnostic laboratory by streaking from the suspension that flow out of diseased tissue into sterile water onto TZC. *R. solanacearum* appears as mucoid, whitish colonies that produce a reddish-pink diffuse pigment (it does not diffuse into the medium). Often there is a brown discoloration of the medium around the colonies. It is best to incubate at 28 °C to increase pigment production. This test is not diagnostic for race or biovar.

There are some contaminant bacteria that have mucoid colonies that could create problems if only semi-selective media is relied upon for detection.

3. Water testing:

a. Sampling protocol

Sampling for *R. solanacearum* from natural bodies of water is more reliable when the water temperature is above 15 °C. In order to increase the likelihood for detection, each site should be sampled at several time points to reduce the effects of environmental variation. Protocol from John Elphinstone (CSL, UK):

1) Disposable sterile tubes should be used to collect the water at a depth of 12-16 inches (30-40 cm) and at least 6.6 ft (2m) from the bank. The tube should be filled after it has reached the sampling depth. Only use shatter proof tubes; do not use glass tubes.
2) At a given site, sampling should occur at 3 places with 2 replicas each (6 samples total) at 9.8 ft (3m) intervals (1a, 1b, 2a, 2b, 3a, 3b).
3) Label each tube with the sample location, date, and sample ID number.
4) Survey each sample location a minimum of 4 times during the season.
5) Store samples in the dark between 4 °C and 10 °C. Samples should arrive at the testing facility within 24 hours.

b. Testing protocol

The sensitivity of this method is limited. The detection level (sensitivity) of this protocol is 10^3 cfu/liter. Bacteria may however be present at lower levels. Competing saprophytic bacteria from the water also limit detection on SMSA medium. Protocol from John Elphinstone:

1) For each tube adjust the sample volume to 50 ml.
2) Centrifuge the samples for 15 minutes at 7000 g at 4-10 °C. Discard the supernatant. For each sample use 1 ml of sterile phosphate buffer (10mM) to re-suspend the pellet.
3) Spread 50-100 µl of the re-suspended pellet on a plate of SMSA medium to isolate. Perform an approximate 1:9 dilution by using the same spreader without flaming on a second plate. Make 2 new plates for each sample.
4) Invert the plates and incubate at 28 °C for 3-5 days. Check each plate for *R. solanacearum* colonies (other bacterial colonies are likely to be present as well).

5) Colonies that are suspected of being *R. solanacearum* undergo further testing.
6) For all suspect isolates perform the tomato host test.

4. Serological test kits: Below is a listing of USDA-APHIS-PPQ-CPHST approved serological test kits: *R*s ImmunoStrip Test, Agdia, Inc., USA; Potato Brown Rot Pocket Diagnostic, Central Science Laboratory (CSL), UK; *Ralstonia solanacearum* SPOT√CHECK LF^{TM}, Scotland.

These serological tests will identify the pathogen at the species level (i.e., *Ralstonia solanacearum*), not at the subspecies level (i.e., race 3). The detection level (sensitivity) of this test is $10^{5 \text{ or } 6}$ cfu/liter.

5. PCR and Real-Time PCR analysis

If a positive result of *Ralstonia solanacearum* is received from the serological test kit, the sample (plant material or culture) must be submitted to USDA-APHIS-PPQ-CPHST for confirmation to race and biovar. At APHIS's request, additional confirmation can be made at the NPDN Regional Centers at Land Grant Universities. The regional centers will have all necessary authorizations to receive samples submitted for identification to the race and biovar level. Real-time PCR analysis generally uses a variation on a protocol that amplifies both a DNA fragment present in all *R. solanacearum* strains and one that is largely Rsr3bv2-specific (Weller et al., 2000). There are also conventional PCR assays that amplify both a "universal" *R. solanacearum* fragment and one that is found only in R3bv2 strains (Fegan et al., 1998).

6. Differentiation of biovars of *R. solanacearum*

Biovars of *R. solanacearum* can be differentiated based on acidification of a carbohydrate panel (Denny and Hayward, 2001). This is a simple and reliable assay and it could be used routinely in combination with other type of methods in diagnostic labs.

Identification to Rsr3b2 should be based on at least two independent methods (i.e. PCR and biovar tests). In addition to tomato inoculations to determine pathogenicity, DNA sequencing analysis may be necessary for confirmation. There is a great need to replace the SMSA plating method and serological tests with economical, easy to use, robust, rapid, highly sensitive and specific method(s) that will be effective for monitoring this pathogen at both the species and subspecies levels.

V. USDA Pathogen Permits and Regulations

USDA/APHIS/PPQ permit and registration requirements for plant diseases and laboratories fall under two authorities, the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). Laboratories receiving suspect infected plant material or cultures are required to have PPQ permits. Laboratories possessing, using, or transferring Select Agents such as Rsr3b2, are required to be registered; however, diagnostic laboratories that identify select agents from a suspect sample are exempt from this requirement as long as they complete an APHIS/CDC Form 4 and destroy the culture within 7 calendar days.

The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status, shipped interstate and require

that the receiving laboratory has a permit. For further guidance on permitting of plant pest material, consult the PPQ permit website at: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ Permit Services at (301) 734-8758.

The Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331) specifies the requirements for possession, use, and transfer of organisms listed as Select Agents such as Rsr3b2. Once an unregistered diagnostic laboratory identifies or suspects a Select Agent, they must immediately notify the APHIS Select Agent Program, complete an APHIS/CDC Form 4 within 7 days, and either destroy or transfer the agent to a registered laboratory within 7 days. In compliance with this Act, if a diagnostic laboratory held back part of a screened sample or culture for voucher purposes and that sample forwarded to the USDA Beltsville Laboratory came back as positive for a Select Agent, the diagnostic laboratory is required to notify the APHIS Select Agent Program immediately. This must take place within seven calendar days of results notification and a PPQ Officer must be provided with the opportunity to witness the destruction of the sample or culture within that time period. Clarification of this and other information related to adherence to the Select Agent regulations is available on the following APHIS website: http://www.aphis.usda.gov/programs/ag_selectagent/index.html, or call (301) 734-5960.

Researchers wishing to work with foreign plant pathogens in the US should review the websites listed above and contact the PPQ permit unit to understand how best to comply with the permitting requirements.

VI. Response

While this plan is focused primarily on recovery, certain aspects of the response to a new detection involve aspects of recovery with a continuum of activities from response to recovery. The response is under USDA, APHIS, Plant Protection and Quarantine's authority delegated from the Secretary under the Plant Protection Act of 2000. After a detection of Rsr3b2 has been confirmed by a USDA, APHIS, PPQ recognized authority, APHIS, in cooperation with the State Department of Agriculture, is responsible for the response. The response may be immediate in the form of advance assessment teams of experts and survey personnel sent to the site of initial detection to place holds, conduct investigations, and initiate delimiting surveys. Further response activities. Actions that may be taken include regulatory measures to quarantine infected or potentially infected production areas, stop the movement of infected or potential infected articles in commerce, and control measures which may include host removal and destruction, or required sanitary practices. APHIS imposes quarantines and regulatory requirements to control and prevent the interstate movement of quarantine significant diseases or regulated articles and works in conjunction with states to impose these actions parallel to state regulatory actions which restrict intrastate movement.

For a confirmed detection of Rsr3b2 in geraniums, the current guidelines prescribe holds at production facilities, traceback and traceforward investigations, and required destruction of infested, or potentially infested, varieties or lots. All host material associated with these lots by shared water irrigation systems or unsanitary greenhouse practices, are also candidates for destruction. All production areas, greenhouse articles, soil, and water systems must be disinfected according to agency guidelines prior to the release of a facility or growing area.

Confirmed infestations by Rsr2b2 of potato or other solanaceous crops will require quarantines of fields, seed tubers, seedlings, or other plant material associated with infested lots, including processing facilities, storage bins, means of conveyance, soil, and irrigation water. Host destruction is required along with disinfection, water decontamination, and several years of non-host production in infected fields or associated growing areas before quarantines can be removed.

VII. Economic Impact and Compensation

Rsr3b2 is a serious pathogen that causes brown rot, or bacterial wilt, of potatoes (Hayward, 1991; Janse, 1996). Bacterial wilt of potato has been estimated to affect 3.75 million acres in approximately 80 countries with global damage estimates exceeding $950 million per year (APHIS, PPQ Action plan, 2004). The bacterium is adapted to cooler temperatures and could be particularly damaging to potato production regions of the US. While race 1 causes losses to tomato and other crops in Florida and the southeastern US (Momol et al., 2003), the potential economic impact of potato brown rot caused by Rsr3b2 is unknown in temperate climates such as where much of US potato production occurs. The actual potential of this race to cause losses in temperate climates is uncertain because data on yield reductions are limited. The successful quarantine and eradication efforts in potato fields in the UK, the Netherlands and Sweden make yield loss estimates difficult. However, in general losses are limited by cool temperatures. When Rsr3b2 is found in potato fields in tropical and subtropical environments without harsh winters, losses can be severe.

As mentioned above, Rsr3b2 is listed as a Select Agent in the Agricultural Bioterrorism Protection Act of 2002. Current authority under the Plant Protection Act only allows for compensation under an Extraordinary Emergency declaration by the Secretary of Agriculture. The current policy does not allow the Risk Management Agency of USDA to pay for undamaged plants. Therefore, any commingled and/or uninfected plants could be destroyed without compensation. This aspect created significant economic losses to geranium producers in the past. Lack of financial compensation for producer losses has already reduced the number of wilted geranium samples submitted to clinics for diagnosis (Personal observation of K. Rane and T. Momol). For infected plants, the adjuster must see the plants but since there are no known effective commercial control measures, losses due to Rsr3b2 (due to destruction orders) are covered causes of loss if the producer followed good farming practices.

VIII. Mitigation and Disease Management

Any disease mitigation strategy that is used should be coordinated with Federal, State and local regulatory officials.

Chemical control: The direct control of brown rot or bacterial wilt diseases caused by *R. solanacearum* is difficult, because of the wide host range, the latency of the pathogen and lack of adequate chemical treatment. Irrigation or effluent water could be treated effectively with low doses of chlorine or paracetic acid. Soil fumigation with vapam, methyl bromide, or chloropicrin is of limited efficacy and utility. A plant-derived volatile compound thymol was found to effectively reduce bacterial wilt incidence on tomato when used as pre-plant soil fumigation (Ji et al., 2005, Momol et al., 2003), however, it's utility as an eradicant has not been evaluated.

Some materials for surface disinfection are listed at
http://www.aphis.usda.gov/ppq/ep/ralstonia/index.html.

Biological control: A number of soil bacteria and plant growth promoting rhizobacteria (PGPR) are currently being investigated for their role in the control of *R. solanacearum*, however, none are currently available commercially and efficacy of the biological controls has yet to be determined.

Cultural control: A number of cultural practices can be employed to reduce disease; overall these are the best means of control at present. These include using healthy (tested) seed potatoes, early detection, accurate identification and reporting of the pathogen quarantine measures on infected fields and farms, sufficient crop rotation, use of cover crops and other measures to reduce the impact of weed hosts and volunteer plants (and in some cases of nematodes), avoidance or testing and treatment of surface water for irrigation, and education. Integrated application of these strategies is key to successful management of diseases caused by *R. solanacearum*.

Germplasm: The best that normal breeding has achieved for most solanaceous crops is moderate level of host resistance to bacterial wilt on a regional level when conditions are not excessively hot or wet. Some potato cultivars are less susceptible to bacterial wilt at least on some regions. There are active potato resistance breeding programs, some of which are focusing on resistance to latent infection. Seven genotypes from two wild Andean potato species were found to have high levels of resistance to wilt and tuber infection in recent years, which provide new resistance sources for developing commercial resistant potato cultivars (CIP, 2004). Three cultivars of eggplant resistant to bacterial wilt have been used in India (Gopalakrishnan et al., 2005)

Extensive international research has produced some highly resistant tomato breeding lines, such as Hawaii 7996, but the resistance is usually linked with undesirable traits like small fruit size. Some large-fruited resistant tomato cultivars (e.g. FL7514) have become commercially available in recent years, and provide moderate resistance against bacterial wilt. Application of acibenzolar-S-methyl enhances host resistance of moderately resistant tomato cultivars (e.g. FL7514) against *R. solanacearum* race 1 biovar 1 (Pradhanang et al., 2005).

Other controls: In offshore geranium production facilities, use of best management practices to exclude the pathogen (see above) and regular testing provides good control when carefully followed by the exporting company. Offshore training efforts could be enhanced by providing funding through USDA, FAS and/or USAID to U.S. scientists specialized on the outreach of *R. solanacearum.*

IX. Current Infrastructure, Needs and Experts

Studies for initial screening of suspect samples can be carried out by triage laboratories (the State's or cooperating university diagnostic laboratory). This screening includes both serological testing methods and isolation and identification of *R. solanacearum* to genus and species. If these facilities cannot perform these screening steps, samples can be referred to the appropriate NPDN Regional Centers located in California, Florida, Hawaii, Kansas, Michigan, New York, and Oregon. If a positive result is obtained through initial screening, confirmatory testing by an approved laboratory to race and biovar will be required.

If the serological test kit gives a positive result of *R. solanacearum*, the sample (plant material, water or soil samples, and/or culture) must be submitted to USDA-APHIS-PPQ-CPHST in Beltsville, MD for determination of race and biovar. This laboratory has all necessary authorizations to receive samples submitted for identification to the race and biovar level. At APHIS's request, additional race and biovar determinations can be made at the NPDN Regional Centers. The regional centers and a few research labs in US have all necessary permit authorizations to receive samples submitted for identification to the race and biovar level.

The infrastructure to handle research on Rsr3b2 is currently very limited due to its Select Agent status. To conduct experiments with this pathogen, registration is required with the USDA-APHIS. Registration is approved on a site-specific basis, taking into account geographical location, research objectives (i.e. if plant inoculation will be conducted), security measures, and a variety of other factors. In addition, the possession, use, or transfer of Rsr3b2 requires entity (institutional) registration. Permits and advance permission are required for any movement of cultures or infected plant materials. These regulations necessarily constrain research progress.

Plant pathologists specialized in *Ralstonia solanacearum* research would benefit from increased funding for research related to Rsr3b2, especially concerning the research priorities listed in the next section. *The destructive nature and quarantine status of this pathogen has heightened its importance and the need for effective detection and management, and for better understanding of this disease and its causal agent.*

***R. solanacearum* Experts:**

J. G. Elphinstone, Phytobacteriologist, specializing in diagnostics, risk assessment and management of quarantine bacteria including *Ralstonia solanacearum*. Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK Phone: +44 (0)1904 462334 Fax: +44 (0)1904 462111. E-mail: j.elphinstone@csl.gov.uk

D. W. Gabriel, Molecular plant pathology, University of Florida, Gainesville, FL, USA

J. B. Jones, Plant Bacteriology, University of Florida, Gainesville, FL, USA

M. T. Momol, Disease management and epidemiology, University of Florida, Quincy, FL, USA. E-Mail: tmomol@ufl.edu

X. Research, Extension, and Education Priorities

Research Priorities -- Most important

1) Develop disease management tactics to control bacterial wilt on potato, tomato and geraniums (Race 1 strains could be used as a model to advance this area), including:
   - Develop or screen additional chemical and biological control products
   - Exclude the pathogen from potato seeds, geranium cuttings, and tomato transplants and develop vegetative plant material certification schemes
   - Study the effects of cover crops, crop rotation and mulches on pathogen dynamics and disease incidence

2) Develop novel methods (i.e., nanotechnology) for rapid, sensitive and accurate detection of *R. solanacearum* at the race and biovar level in the environmental samples (including host plant, water, soil, and other media). Serological test kits (i.e., immunostrips) do not yet have the requisite sensitivity or specificity for low-level latent infections or water samples or race/biovar determination, but are excellent for diagnosis of full-blown disease on symptomatic plants. ELISA may be more sensitive, but is more technically demanding and time-consuming.
3) Develop additional DNA and serology based detection tools that can be used reliably to distinguish subgroups, races, and biovars of *R. solanacearum*, especially Rsr3b2 from other endemic strains of *Ralstonia*.
4) Develop a better understanding of latent infections by *R. solanacearum* because these are an important source of potential pest introductions on potato and geranium. Research is needed to understand what factors predispose plants to be latently infected, how bacteria move in latently infected plants, and what triggers symptom development in latently infected plants.
5) Develop and enhance bacterial wilt resistant potato and tomato cultivars.

Research Priorities -- Highly important

1) Develop disease-resistant tomato rootstock together with efficient (preferably robotic) grafting techniques for seedling production
2) A broader host range study should be done to determine which commonly grown ornamentals and other plant species act as hosts or carriers of *Ralstonia solanacearum* race 3 biovar 2.
3) Explore novel diagnostic techniques to detect R3b2 quickly and at low concentrations, such as electronic nose and highly sensitive antibody-responsive cell culture systems, etc.
6) Determine if any other races (or subgroups) / biovars of *Rs* besides r3b2 have cold tolerance capability. What gene sequence(s) code for cold tolerance known? This knowledge might allow regulations directed against all strains of *Rs* with the sequence(s).
7) Continue the work on cold tolerance of Rsr3b2 strains including those from Guatemala, Mexico, Costa Rica, Kenya, and China to see if they can survive in temperate US locations.

Research Priorities -- Important

1) Methods that can be used to disinfest contaminated soils, including soil fumigation, other chemicals and biologicals. Testing of other area sanitizers for site clean-up (such as uses of quats and peroxyacetic acid sanitizers with foam carriers, Virkon S, maybe some others).
2) Determine a practical disposal method for potatoes in the event that detection of the bacterium in potato growing areas and find it necessary to quarantine fields.
3) Develop transgenic tomato and potato resistant to the disease.

Education Priorities

1. Educate a new cadre of plant pathologists in the epidemiology and management of bacterial diseases.

2. Develop training courses on detection, monitoring, and management of *Ralstonia* related diseases.

Extension Priorities

1. Educate county extension, growers and crop advisors in sampling, monitoring and management of related diseases and in the utility of map-based tracking and information systems such as the Pest Information Platform for Education and Extension (PIPE).
2. Develop contingency plans to test potato propagation material to ensure that it is free of Rsr3b2. Existing EU-EPPO protocols for such testing should be used as a starting point.
3. Survey tomato, potato, and other host growing areas and greenhouse areas in the U.S. with previous Rsr3b2 history, including soil, water, crops and weed samples.

References

CIP, 2004. Breakthrough in bacterial wilt resistance. International Potato Center Annual Report 2004. WWW.CIPOTATO.ORG/MARKET/ARS/AR2004/AR2004_06.HTM [accessed February 2006]

Daughtrey, M. 2003. New and re-emerging diseases in 2003. Department of Plant Pathology, Cornell University, Long Island Horticultural Research & Extension Center.

Denny, T. P. and Hayward, A.C. 2001. Ralstonia, pages 151-174 in: Schaad, N. W. et al. Laboratory guide for the identification of plant pathogenic bacteria, 3rd ed. APS Press, St. Paul, 373 pp.

Fegan, M., Holoway, G., Hayward, A. C., and Timmis, J. 1998. Development of a diagnostic test based on the polymerase chain reaction to identify strains of *R. solanacearum* exhibiting the biovar 2 genotype. Pages 34-43 in: Bacterial Wilt Disease: Molecular and Ecological Aspects. P. Prior, C. Allen, and J. Elphinstone, eds. Springer-Verlag, Berlin.

Fegan, M., and Prior, P. 2005. How complex is the *Ralstonia solanacearum* species complex? Pages 449-461 in: Bacterial Wilt: The Disease and the *Ralstonia solanacearum* Species Complex. C. Allen, P. Prior, and A. C. Hayward, eds. American Phytopathological Society, St. Paul.

Gabriel, D. W, Allen, C., Schell, M., Denny, T. P., Greenberg, J. T., Duan, Y. P., Flores-Cruz, Z. Huang, Q., Clifford, J. M., Presting, G., González, E. T., Reddy, J., Elphinstone, J., Swanson, J., Yao, J., Mulholland, V., Liu, L., Farmerie, W., Patnaikuni, M., Balogh, B., Norman, D., Alvarez, A., Castillo, J. A., Jones, J. B., Saddler, G., Walunas, T., Zhukov, A., and Mikhailova, N. 2006. Identification of Open Reading Frames Unique to a Select Agent: *Ralstonia solanacearum* Race 3 Biovar 2. MPMI 19/1:69-79.

Gopalakrishnan, T. R., Singh, P. K., Sheela, K. B., Shankar, M. A., Kutty, P. C. J., and Peter, K. V. 2005. Development of bacterial wilt resistant varieties and basis of resistance in eggplant (*Solanum melongena* L.). In C. Allen, P. Prior, & A. C. Hayward (Eds.), *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex* (pp. 293-300). St. Paul, MN: APS Press.

Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 29:65-87.

Janse, J. 1996. Potato brown rot in western Europe – history, present occurrence and some remarks on possible origin, epidemiology and control strategies. Bulletin OEPP/EPPO 26: 679-695.

Ji, P., M.T. Momol, S.M. Olson, P.M. Pradhanang and J.B. Jones. 2005. Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. Plant Dis. 89:497-500.

Kim, S. H., Olson, R. N. and Schaad, N. 2002. *Ralstonia solanacearum* Biovar 2, Race 3 in geraniums imported from Guatemala to Pennsylvania in 1999. Plant Dis. 92:S42.

Kim, S. H., Olson, R. N., Schaad, N. W., and Moorman, G. W. 2003. *Ralstonia solanacearum* race 3, biovar 2, the causal agent of brown rot of potato, identified in Geraniums in Pennsylvania, Delaware, and Connecticut. Plant Dis. 87:450.

Liu, H., Kang, Y., Genin, S., Schell, M. A., and Denny, T. P. 2001. Twitching motility of *Ralstonia solanacearum* requires a type IV pilus system. Microbiology-UK 147:3215-3229.
Momol, T., Jones, J., Olson, S. 2003. New outbreak of *Ralstonia solanacearum* (race 3 biovar 2) in geraniums in US and effects of biofumigants on *Ralstonia solanacearum* (race 1 biovar 1), University of Florida Pest Alert on webpage: http://extlab7.entnem.ufl.edu/PestAlert/tmm-0303.htm.

O'Hern, C. 2004 January 5. Detection of *Ralstonia solanacearum* race 3 biovar 2 in New York greenhouse (document by Richard Dunkle) [Distribution list]. Accessed 2004 January 5.

Pradhanang, P.M., Ji, P., Momol, M.T., Olson, S.M., Mayfield, J. L. and Jones, J.B. 2005. Application of acibenzolar-S-methyl enhances host resistance in tomato against *Ralstonia solanacearum*. Plant Dis. 89: 989-993.

Swanson, J. K., Yao, J., Tans-Kersten, J. K., and Allen, C. 2005. Behavior of *Ralstonia solanacearum* race 3 biovar 2 during latent and active infection of geranium. Phytopathology 95:136-143.

Weller, S. A., Elphinstone, J. G., Smith, N. C., Boonham, N., and Stead, D. 2000. Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time fluorogenic PCR (TaqMan) assay. Appl. Environ. Microbiol. 66:2853-2858.

Williamson, L., Nakoho, K., Hudelson, B. and Allen, C. 2002. *Ralstonia solanacearum* race 3, biovar 2 strains isolated from geranium are pathogenic on potato. Plant Dis. 86:987-991.

Web Resources

APHIS, PPQ Action plan (Joel Floyd et al., 2004)
http://www.aphis.usda.gov/ppq/ep/ralstonia/rasltoniaactionplanv4web.pdf

APHIS, PPQ Pest Alert
http://www.aphis.usda.gov/ppq/ep/ralstonia

National IPM Certers. National Pest Alert: *Ralstonia solanacearum race 3 bovar 2*
http://ncipmc.org/alerts/ralstonia.pdf

NPDN *Ralstonia solanacearum* 3/2 SOP (K. Snover et al., 2004)
http://spdn.ifas.ufl.edu/NPDN_SOPs.htm.

Pictorial Guide to Geranium Wilt Disorders (B.E. Whipker 2003)
http://www.ces.ncsu.edu/depts/hort/floriculture/GeraniumWilt.pdf