# Recovery Plan

# for

# *Ralstonia solanacearum* Race 3 Biovar 2

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

May 20, 2010

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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 made and actions 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*, formerly called *Pseudomonas solanacearum*. Historically, strains of *R. solanacearum* were classified into 5 races based loosely on host range, and into 5 biovars based on their differential ability to produce acid from a panel of carbohydrates. Race 1 of the pathogen naturally occurs in tropical and subtropical areas worldwide. In the United States, this race occurs in the southern states, where it causes bacterial wilt on a range of cultivated crops, such as pepper, tobacco, tomato, and rarely, potato. Bacterial wilt caused by this group has not spread to potato elsewhere in the United States because the seed potato system is isolated from the potato production system and because it is thought that the endemic southeastern strains cannot survive in colder areas of the United States. In contrast, *R. solanacearum* race 3 biovar 2 (R3b2, phylotype II, sequevar 1) originated in South American tropical highlands and can survive in temperate climates. In Europe, R3b2 has been responsible for several outbreaks of the disease over the last decades. The ability of R3b2 to survive cool temperature and its aggressiveness on potato make it a serious threat to American agriculture.

Although R3b2 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 and Canada. R3b2 has been accidentally introduced into the U.S. on geranium cuttings produced in the highland tropics of Africa and Central America. Geraniums imported into the U.S. on several occasions in 1999 and 2000 contained R3b2. 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. No positive identification of R3b2 has been reported in the US since 2004 and R3b2 has never been detected outside of these introductions on geranium. In order to determine the identity and source of future R3b2 introductions a forensic capability should be developed involving diverse culture collections and genomic sequencing.

R3b2 causes bacterial wilt, which on potato is also known as brown rot of potato. Brown rot 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 R3b2 is unknown in temperate climates such as those where potatoes are grown in the United States. If introduced, R3b2 could affect a potato crop in the U.S. valued at $3.1 billion in 2009 with an export value of $1.2 billion for a variety of potato products.

R3b2 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 to register these agents with USDA, APHIS Plant Protection and Quarantine.

Management of brown rot caused by R3b2 is difficult due to lack of effective control measures and resistant potato cultivars. Chemical control, such as soil fumigation with vapam, methyl bromide, or chloropicrin is of limited efficacy. Additionally, detection of the pathogen can be difficult due to occurrence of latent (symptomless) infections in potato tubers or in geranium

cuttings. Only a few acceptable tolerant tomato cultivars are available that provide moderate levels of disease control and their efficacy is limited geographically. In Florida, application of thymol, a plant-derived volatile compound, was shown to effectively reduce bacterial wilt on tomato caused by *R. solanacearum* race 1, but its effect on control of R3b2 on potato is unknown. Consequently, best protection from losses to R3b2 in the US will be achieved mainly by exclusion through the effective use of statutory quarantines, effective sanitation standards for off-shore geranium production, early detection, and eradication by host destruction. Development of effective disease management strategies and improvement of detection and monitoring tools are key components of this recovery strategy. Besides these recommended actions other research, education, and extension priorities for effectively mitigating R3b2 are summarized in this report.

**Recommended Actions:**

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 additional DNA and immunological detection tools that can be used reliably to distinguish subgroups of *R. solanacearum*, especially R3b2, from other endemic strains of *Ralstonia*.
3) 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).

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

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## I. Introduction

Bacterial wilt is an important disease of many crops and is caused by various subgroups of the bacterium *Ralstonia solanacearum*, formerly called *Pseudomonas solanacearum*. Historically, strains of *R. solanacearum* were classified into five races based loosely on host range, and into five biovars based on differential ability to produce acid from a panel of carbohydrates (Denny, 2006). There is no general correlation between races and biovars; however biovar 2 strains are usually 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 as well as Asia, Africa, and South America. 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 (Denny, 2006). Recently a more phylogenetically meaningful system has classified *R. solanacearum* into four major genetic groups called phylotypes that reflect the geographical origin and ancestral relationships between strains (Fegan and Prior, 2005). *R. solanacearum* race 3 biovar 2 (R3b2) belongs to Phylotype II (sequevars 1 and 2). 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 this near-clonal subgroup is widely distributed in tropical regions throughout the world and some temperate regions such as Europe and northern Asia.

Several Race 1 groups of the pathogen are established in the southern United States, where they cause bacterial wilt of tomato and other crops, such as pepper, tobacco, and rarely, potato. This race has not spread throughout the United States, in large part due to the seed potato system being isolated from the production system and strains of race 1 not being able to overwinter in northern environments and/or soils with low water holding capacity. In contrast, R3b2 can survive in temperate climates including highland tropics, UK, and the Netherlands (Elphinstone, 2005), usually in association with plant tissue (Milling et al., 2009). Race 3 is highly pathogenic on potato and tomato (primary hosts) and can also infect and eventually kill other solanaceous

plants (e.g. eggplant, nightshade weeds) 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 sequence of UW551 (R3b2 strain) genome was published and led to the identification of R3b2-specific genes (Gabriel et al., 2006). This information will facilitate future Race 3 specific molecular diagnostic assay developments.

## II. Signs and Symptoms

1. Symptoms of the disease:

Foliage on potatoes infected with *R. solanacearum* may be stunted, yellowed, and wilted. Early in the infection process, wilting of leaves may be limited to the top portion of plants and to only an individual branch or leaflet. Infected plants may appear to recover at night when temperatures are cooler, but soon wilting becomes irreversible and death of the plants follows. The stems of young plants may collapse and/or have narrow dark streaks. 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 grey-brown discoloration and ooze (Fig. 1). Initially the vascular ring appears yellow to light brown, but as the infection progresses the ring will become browner (Champoiseau et al., 2009).


**Figure 1.** Symptoms of brown rot caused by *R. solanacearum* race 3 biovar 2 on potato. Photo credits: (A) D. Thurston, Cornell University (A) and P. Champoiseau, University of Florida -IFAS (B).

On tomatoes, symptoms of bacterial wilt caused by *R. solanacearum* are the same whether disease is caused by R3b2 or other groups. Wilt is first seen as a flagging of one or two leaves on plants, usually at the top portions of plants. Under favorable conditions, the disease develops rapidly and may kill plants as quickly as 4-7 days after appearance of the first wilt symptoms (Fig. 2).

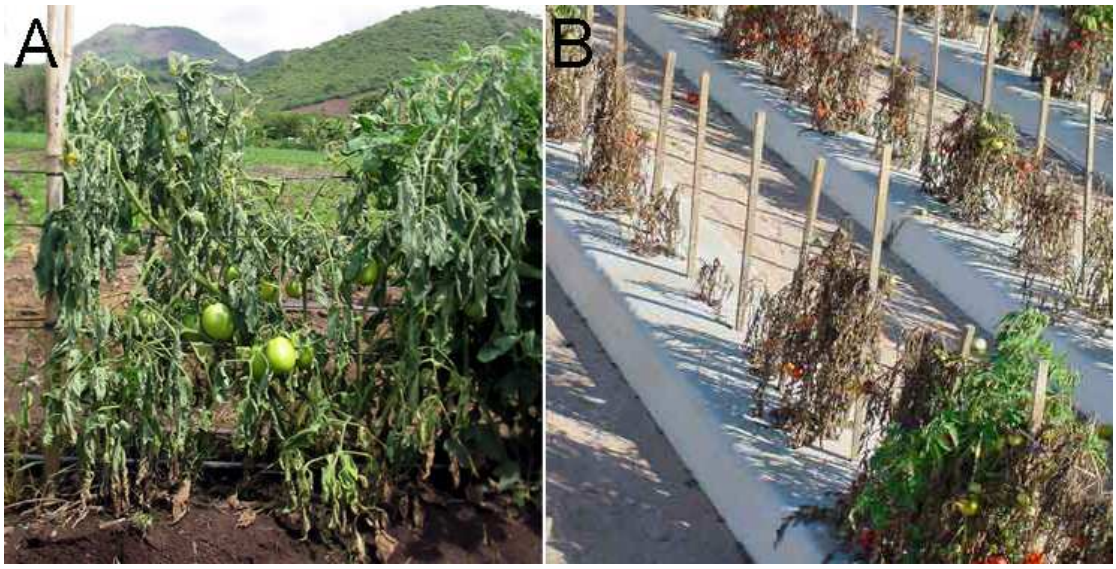


**Figure 2.** Symptoms of bacterial wilt of tomato caused by *R. solanacearum* in Guatemala (A) and Florida (B). Photo credits: C. Allen, University of Wisconsin (A) and T. M. Momol, University of Florida-IFAS (B).

On geranium, the earliest symptom of bacterial wilt can be very subtle and easily overlooked, developing as a leaf scorch in sectors and abnormal yellowing of lower leaves. A characteristic symptom of Southern wilt of geranium is the upward curling of leaf margins (Champoiseau et al., 2009). Soon after these initial symptoms appear, geranium plants begin to express wilt symptoms (Fig. 3).

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

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 produces leaf spots and symptom expression is favored by high temperatures (29-35°C or 85-95°F).

2. Signs of the pathogen:

Bacterial streaming is a common diagnostic sign of *R. solanacearum*. When cut stem sections from infected plants are placed in water, threads of a viscous white slime can be observed streaming from the cut end of the stem within 15 minutes. These threads are bacterial ooze exuding from the infected xylem vascular bundles (Fig. 4). This streaming test is a valuable diagnostic tool for quick detection of bacterial wilt or brown rot in the field (Allen et al., 2001). Bacterial ooze may also emerge from the vascular ring of cut potato tubers. Ooze from intact tubers at eyes or where the stolon attaches may cause dirt to adhere to tubers (Fig. 5). If the vascular tissue has collapsed, sunken skin lesions will also appear. These signs may not be visible early in disease development.

**Figure 4.** Bacterial streaming from freshly cut wilted tomato stem when placed in clear water. Photo credit: University of Georgia, Plant Pathology Extension.


**Figure 5.** Sign of *R. solanacearum* (race 3 biovar 2) on potato: Bacterial ooze from vascular tissues. Photo credit: P. Champoiseau, University of Florida-IFAS.

3. Latent infections:

Under favorable conditions, plants infected with *R. solanacearum* may not show symptoms; this is termed a latent infection and is common in both potato and geranium (Swanson et al., 2005). Latent infections are of major importance in spread of *R. solanacearum* and epidemiology of the disease. It was shown that latently infected geranium cuttings were responsible for introduction of R3b2 to the U.S. in 2003 and 2004.

4. Look-alike symptoms:

Other plant pathogens, such as *Fusarium oxysporum* f. sp. *lycopersici* (Fusarium wilt on tomato), *Verticillium* wilt on potato, root damage, drought, or nutrient deficiency can incite symptoms similar to those symptoms as those associated with bacterial wilt caused by *R. solanacearum*; therefore, symptoms alone should not be used for diagnosis. For this reason, caution is needed in determining if a symptomatic plant really has the disease. This is especially true for bacterial wilt caused by R3b2, since similar symptoms in different plants may be caused by race 1 of the pathogen which is endemic in the southern United States. Furthermore, the current commercial ELISA or immunostrip tests will react with both R3b2 and endemic Rs race 1.

## III. Spread and Risk Map

*Ralstonia solanacearum* is primarily a soilborne and waterborne pathogen and 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). In greenhouses, it may also be spread by transplanting infected plants, taking cuttings without disinfecting grafting knives between plants, pinching buds of plants, and especially by irrigating with sub-irrigation or ebb-and-flow systems. *R. solanacearum* primarily infects host plants through their roots by entering through wounds formed by lateral root emergence or by root damage caused by handling or soilborne organisms (e.g. the root-knot nematode). The bacterium can also enter plants by way of stem injuries from insects, handling, or tools. The pathogen does not readily spread from plant-to-plant through the splashing of water, casual contact, or aerially (Swanson et al., 2005). Greenhouse spread is easy to control by application of exclusionary and phytosanitary practices including avoidance of ebb-and-flow and flooding irrigation systems.

R3b2 has entered the U.S. through the introduction of latently infected geranium cuttings produced in greenhouses located outside the country. On several occasions in 1999 and 2000, imported geranium cuttings were positive for R3b2 (Kim et al., 2002 and 2003, Williamson et al., 2002). During 2001 and 2002 the U.S. had no reported cases of R3b2. 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. State and APHIS personnel worked 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).

A risk map for R3b2 and bacterial wilt was created using NAPPFAST (Magarey et al., 2007) at the Center for Plant Health Science and Technology of the USDA APHIS to depict the potential areas that are suitable or highly suitable for growth and establishment of the pathogen on a scale of 1-10 (see *Appendix 1*). The risk map is a combination of a host density map, which represents the potential area of growth and establishment of the pathogen based on primary and secondary host acreages, and a climatic map, which describes the relative climatic suitability for R3b2 to grow and survive, built-up at the county level. A value of 1 represents low density of susceptible hosts and low likely of pest growth and survival. A value of 10 indicates high density of susceptible hosts and a likelihood of pest growth and survival.

## IV. Detection and Identification

Confirmatory diagnosis of bacterial wilt caused by R3b2 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 [accessed August 10, 2009].

1. Diagnostic screening tests:

*a. Bacterial streaming.*

Clouds of bacteria usually appear within 20 minutes when petioles or stems of the suspected plants are placed in tubes of sterile water as described above (Fig. 4). 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. This streaming test is a valuable diagnostic tool for quick detection of bacterial wilt or brown rot in the field.

*b. Serological test kits.*

Symptomatic plants in the field or in the greenhouse can be tested for *R. solanacearum* with immunodiagnostic assays using species-specific antibodies. The USDA-APHIS-PPQ has tested and recommends the use of commercially-available immunostrips for rapid detection of *R. solanacearum* in the field or lab: *Rs* ImmunoStrip Test, Agdia, Inc., USA; Potato Brown Rot Pocket Diagnostic, Central Science Laboratory (CSL), UK; *Ralstonia solanacearum* SPOT√CHECK LF™, Scotland (Floyd, 2007).

Screening tests are cheap, fast and require minimum equipment. However, they cannot be used to identify the "race" or biovar and can only detect high populations of the pathogen. The detection level (sensitivity) of these tests is $10^5$ colony forming units (cfu).

2. Isolation, culture and identification:

*a. Water, plant and soil sampling.*

With experience, *R. solanacearum* can be isolated and cultured from diseased tissue, and soil and water samples. Optimized sampling protocols from USDA-APHIS (Floyd, 2007) and the European Union (EU Commission Directive 2006/63/CE, 2006) should be used for isolation of the bacterium.

Sampling 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.

*b. Use of semi-selective medium (SMSA).*

The bacteria may be cultured from diseased tissue, soil and water samples in a diagnostic laboratory by streaking from the suspensions that flow out from diseased tissue, soils, or water samples into sterile water onto Petri plates containing SMSA semi-selective medium (Elphinstone et al., 1996). SMSA medium contains antibiotics and fungicides that will inhibit or reduce growth of competing saprophytic bacteria or fungi. The plates should be incubated at 28 °C for 3-5 days. Virulent colonies of *R. solanacearum* appear as small mucoid, irregular, whitish colonies with pink color in the center after 48-60 hours (Fig. 6). The colonies then develop blood red whorls in the center after further incubation. Most colonies that are not *R. solanacearum* will appear red throughout. However some contaminant bacteria form similar mucoid colonies that could create problems if only semi-selective media is relied upon for detection. This test is not diagnostic for race or biovar.

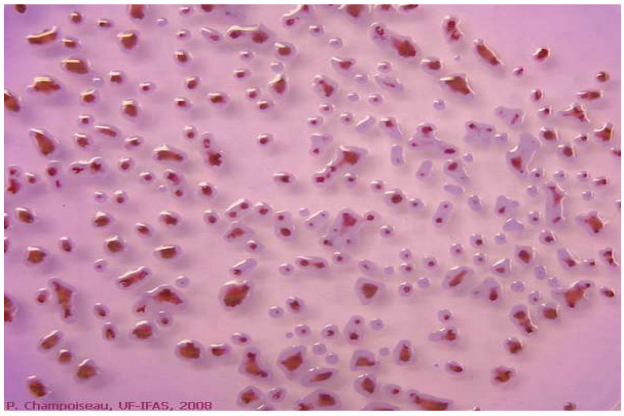


**Figure 6.** Virulent colonies of *R. solanacearum* on modified SMSA medium after 4 days. Photo credit: P. Champoiseau, University of Florida-IFAS.

*c. Other culture media.*

Other media can be used to retrieve *R. solanacearum* from liquid or frozen stock suspensions or for routine culture of the bacteria (Denny and Hayward, 2001). A tetrazolium chloride medium (TZC) can help to differentiate colonies of the normal or virulent type, which are white or cream-colored, irregularly shaped, highly fluidal, and opaque, from colonies of the mutant or non-virulent type which appear uniformly round, smaller, and butyrous (or dry). It is important to note that *R. solanacearum* is a slow-growing microbe even on this rich medium. Colonies that are visible in less than 36h at 28C are not *R. solanacearum*. Non virulent colonies are favored by long-term storage. 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.

*d. Twitching motility.*

It is helpful to observe the microscopic colonies at 24 to 36 hours on solidified rich growth media (i.e. SMSA, TZC) for twitching motility. Twitching motility is a form of translocation of bacteria over solid surfaces involving Type IV pili system. 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).

3. Phylotype and biovar identification:

*a. DNA-based methods.*

Race determination is not possible, because *R. solanacearum* strains do not have race-cultivar specificity on plant hosts and, with the exception of R3b2, the old "races" do not have phylogenetic unity. However PCR and Real-Time PCR assays that use phylotype or biovar-specific primers can be used for identification of *R. solanacearum* phylotype II or biovar 2 strains (Fegan and Prior, 2005; Weller et al., 2000). 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 R3b2-specific (Weller et al., 2000). A conventional PCR assay can amplify both a 280 bp "universal" *R. solanacearum* fragment (primer pair 759/760) (Fegan and Prior, 2005) and one that is found only in R3b2 strains (630/631) (Fegan et al., 1998). Strains of *R. solanacearum* can be sub-classified into phylotypes with a single multiplex PCR reaction and then into sequevars using PCR amplification and gene sequence analysis of the conserved endoglucanase (*egl*) gene (Fegan and Prior, 2005).

*b. Differentiation of biovars.*

Biovars of *R. solanacearum* can be differentiated based on differential ability of strains to acidify culture media containing a panel of carbohydrate substrates (Denny, 2006). This is a simple and reliable assay can be used routinely in combination with other methods in diagnostic labs (Fig.6).

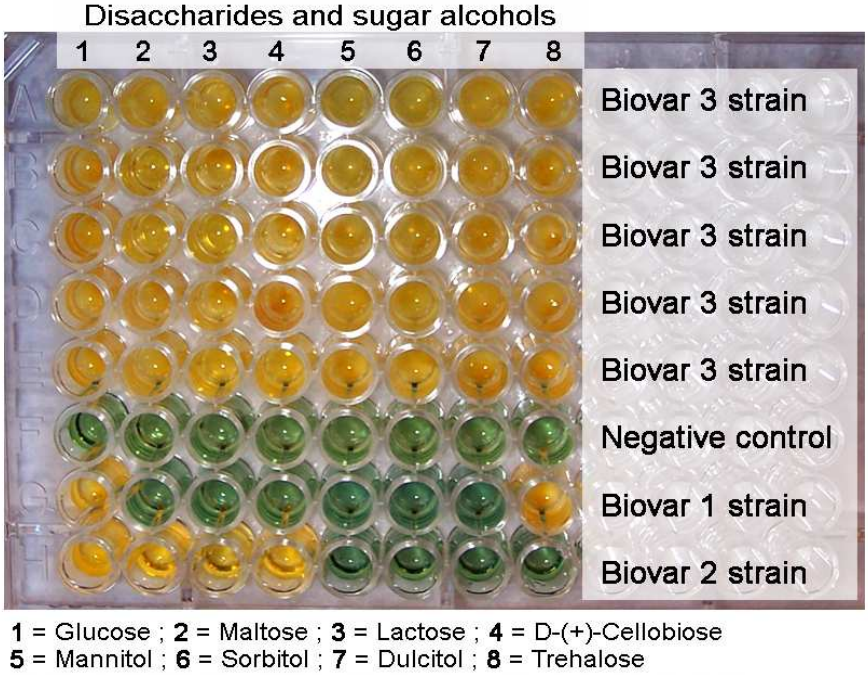


**Figure 6.** A microtiter plate showing the results of a biovar test with seven strains of *R. solanacearum*. Photo credit: E. N. Twieg, USDA-APHIS-PPQ-CPHST-National Plant Germplasm and Biotechnology Laboratory, Beltsville, MD.

**Identification to R3b2 should be based on at least two independent methods, including the biovar test and one of the DNA-based methods (i.e. PCR or real-time PCR). 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. Any suspected R3b2 samples must be forwarded to USDA-APHIS-PPQ laboratories in Beltsville, MD for regulatory confirmation.**

## 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 R3b2, are required to be registered; however, diagnostic screening 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(s) within 7 calendar days (Floyd, 2007).

The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status, that when shipped interstate require the receiving laboratory to have a permit. For further guidance on permitting of plant pest material, consult the PPQ permit website at: http://www.aphis.usda.gov/ppq/permits/ [accessed August 11, 2009] or contact PPQ Permit Services at 301-734-0841.

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 R3b2. Once an unregistered diagnostic laboratory identifies or suspects a Select Agent, they must immediately notify the APHIS Select Agent Program (within 24 hours of confirmation), complete an APHIS/CDC Form 4 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 7 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.shtml [accessed August 11, 2009] , or call APHIS Select Agent Program 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 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 R3b2 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 with advance assessment teams and survey personnel sent to the site of initial detection to place holds, conduct investigations, and initiate delimiting surveys. Further actions may include regulatory measures to quarantine infested or potentially infested production areas, stop the movement of infested or potential infested articles in commerce, and control measures such as host removal and destruction, or other required phytosanitary 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 R3b2 in geraniums, the current new pest response guidelines (APHIS-PPQ, 2005) prescribe holds at production facilities, traceback and traceforward investigations, and required destruction of infested and potentially infested geranium 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 disinfested according to the guidelines prior to the regulatory release of a facility or growing area.

Confirmed infestations by R3b2 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. More details on response procedures to confirmed identification of R3b2 in the US can be found in the USDA-APHIS-PPQ's New Pest Response Guidelines (Floyd, 2007).

# VII. Economic Impact and Compensation

*R. solanacearum* R3b2 is one of the most damaging pathogens on potato worldwide (Hayward, 1991; Janse, 1996). Brown rot of potato has been estimated to affect 3.75 million acres in approximately 80 countries with global damage estimates exceeding $950 million per year (Floyd, 2007). The bacterium is adapted to cooler temperatures and could be particularly damaging to potato production regions of the northern U.S. While "race 1" causes losses to tomato and other crops in Florida and the southeastern U.S. the potential economic impact of potato brown rot caused by R3b2 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 due to quarantine and eradication efforts in potato fields in the UK, the Netherlands and Sweden. Despite widespread detection of the pathogen in northern Europe, direct losses of potato to the disease have been limited to a few

outbreaks during unusually hot summers (Priou et al 2006). Nonetheless the economic impact of the additional quarantine-related testing has been significant.

As mentioned above, R3b2 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 has caused significant economic losses to geranium producers in the past.

# VIII. Mitigation and Disease Management

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

1. Control on potato and tomato:

Because *R. solanacearum* is a soilborne pathogen and host resistance is limited, bacterial wilt is very difficult to control in field production on crops such as potato and tomato (Hayward, 1991; Saddler, 2005). Moreover, *R. solanacearum* is very widely distributed and has an unusually broad host range (Denny 2006). Thus, no single strategy is 100% effective in control of the disease. However, in locations where the pathogen is established, some level of bacterial wilt control is possible by using a combination of diverse control methods. These methods should be used as part of an integrated management strategy, and include:

*a. Host resistance.*

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 in 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 and BHN 466) have become commercially available in recent years, and provide moderate resistance against bacterial wilt. Grafting susceptible tomato cultivars onto resistant tomato or other solanaceous rootstocks is effective against Asian strains of *R. solanacearum* and is used on the commercial scale in different locations worldwide (Saddler, 2005). However, effectiveness of grafting for use against R3b2 has not been tested.

*b. Chemical control.*

Direct control of brown rot or other bacterial wilt diseases caused by *R. solanacearum* in the field is difficult, because of the wide host range, strain variation, and lack of adequate chemical treatments. Chemical controls for bacterial wilt that have been show to have both greenhouse and to a lesser extent field efficacy include; Actigard (e.g., acibenzolar-*S*-methyl) and phosphorous acid (Anith et al., 2004, Ji et al., 2007, Norman et al., 2006, Pradhanang et al., 2005). These chemical controls all need to be validated on a larger scale and against R3b2. Irrigation or effluent water could be treated effectively with low doses of chlorine or peracetic acid. Soil fumigation with vapam, methyl bromide, or chloropicrin is of limited efficacy and utility. The 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); however, its 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 [accessed May 10, 2010].

*c. 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* in small scale experiments; however, none are currently available commercially and efficacy of the biological controls has yet to be determined on a commercial scale.

*d. Phytosanitation and cultural practices.*

The best strategy for controlling bacterial wilt in the field consists primarily of phytosanitation and cultural practices. In regions where bacterial wilt of potato is endemic or in locations where *R. solanacearum* is present but not yet established, these methods can be effective under some conditions. A number of cultural practices can reduce disease, including planting 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. Recommended strategies for best management of bacterial wilt of tomato caused by *R. solanacearum* should be followed (Momol et al., 2005). Integrated application of these strategies to other crops is critical for successful management of diseases caused by *R. solanacearum*.

*e. Exclusionary practices.*

In locations where the pathogen is not present, it is important to prevent introduction and, if inadvertently introduced, to prevent subsequent movement of race 1 of *R. solanacearum* from infested to healthy locations or fields. Effective cultural sanitation practices are critical to keep non-infested areas clean. Sanitation efforts include planting only certified disease-free plantlets, disinfesting all equipment before moving it between fields, controlling floodwater flow, and never using surface water for irrigation. In the greenhouse, sanitary practices for tomato transplant production may include avoidance of sub-irrigation, wide separation of greenhouses from field production areas, disinfestations of all frames, trays and tools, use of pathogen-free soils or potting mix, control of weeds, and limited handling of plants (Mc Carter, 1991).

2. Control on geranium:

In offshore geranium production facilities, USDA-APHIS requires use of sanitation protocols to exclude the pathogen (APHIS-PPQ, 2005). Exclusionary practices, along with regular testing and personnel training provide 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 in outreach on *R. solanacearum.*

## IX. Current Infrastructure, Needs and Experts

Studies for initial screening of suspect samples can be carried out by diagnostic laboratories (private, state, NPDN or cooperating university diagnostic laboratories). 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 biovar and identification by PCR methods is required.

If the serological test kit gives a positive result for *R. solanacearum*, the sample (plant material, water or soil samples, and/or culture) must be submitted to USDA-APHIS-PPQ-CPHST-NPGB Laboratory in Beltsville, MD for testing for R3b2. This laboratory has all necessary authorizations to receive samples submitted for identification. At APHIS's request, additional determinations can be made at the NPDN Regional Centers. The regional centers and a few research laboratories in the US have all necessary permit authorizations to receive samples submitted for identification to determine if they are R3b2.

The infrastructure to handle research on R3b2 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 R3b2 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.

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. For this purpose a team of experts on *R. solanacearum* from the universities of Florida, Georgia, Hawaii, and Wisconsin was granted a 4-year (2007-2011) USDA-NRI funded project to conduct research on *R. solanacearum* R3b2. Objectives of the project are *i)* to develop rapid, robust, and reliable diagnostic assays for *R. solanacearum* race 3 biovar 2, *ii)* to identify R3b2 genes involved in cold adaptation and growth in plant hosts, using a microarray-based post-genomic approach, and *iii)* to develop a package of optimized education and management training modules that will educate stakeholders to control this pathogen, primarily by exclusion. More details about this project (summary, contact information, and accomplishments) can be found at http://plantpath.ifas.ufl.edu/rsol/NRI_Project/Projectsummary.html [accessed May 10, 2010].

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# 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 additional DNA and immunological detection tools that can be used reliably to distinguish subgroups of *R. solanacearum*, especially R3b2, from other endemic strains of *Ralstonia*.
3) 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.
4) Determine if any other subgroups of *Rs* besides R3b2 have cold tolerance capability. What gene sequence(s) code for cold tolerance? This knowledge might allow regulations directed against all strains of *Rs* with the sequence(s).
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* R3b2.
3) Develop novel methods (i.e., use of nanotechnologies, isothermal DNA amplifications) for rapid, sensitive and accurate detection and identification to subgroups of *R. solanacearum* in environmental samples (including host plant, water, soil, and other media).
4) 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.
5) Continue the work on cold tolerance of R3b2 strains including those from Guatemala, Mexico, Costa Rica, Kenya, and China to see if they can survive in temperate US locations.
6) Develop rapid and cheap gene sequencing methods for identification of sequevars of *R. solanacearum*. Create a database including all partial gene sequences that allow differentiation of the sequevars.

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 quaternary ammonium and peroxyacetic acid sanitizers with foam carriers, Virkon S, maybe some others).
2) Determine a practical disposal method for potatoes in the event of detection of the bacterium in potato growing areas and/or subsequent quarantine.
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 R3b2. 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 R3b2 history, including soil, water, crops and weed samples.

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## Web Resources

***Ralstonia solanacearum*/Brown rot-Bacterial wilt website (online since September 2008):**
http://plantpath.ifas.ufl.edu/rsol/

**USDA-APHIS permits webpage:**
http://www.aphis.usda.gov/plant_health/permits/index.shtml

**USDA-APHIS Select Agents and Toxins list:**
http://www.aphis.usda.gov/programs/ag_selectagent/ag_bioterr_toxinlist.shtml

**Minimum sanitation protocols for offshore geranium cutting production**
http://www.aphis.usda.gov/plant_health/plant_pest_info/ralstonia/downloads/ralstoniaworkplan.pdf

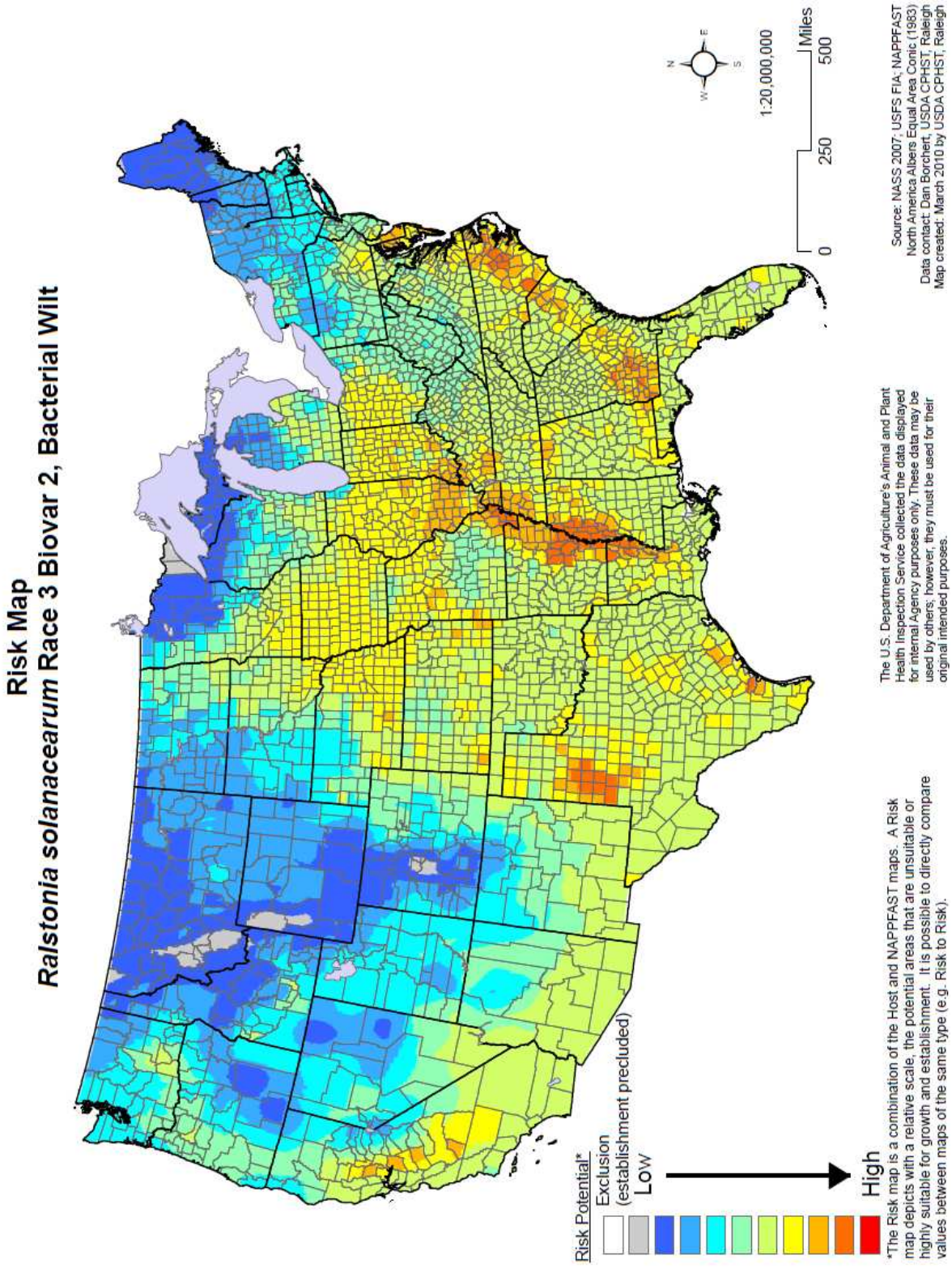
**Optimized protocols for detection and identification of *R. solanacearum* - European Union Council Directive 98-57-EC**
http://plantpath.ifas.ufl.edu/rsol/RalstoniaPublications_PDF/Protocols_UE_CoucilDirective_98-57-EC_2006.pdf

**New pest response guidelines: *Ralstonia solanacearum* race 3 biovar 2**
http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/nprg-ralstonia.pdf

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

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

Appendix 1


Risk Map
Ralstonia solanacearum Race 3 Biovar 2, Bacterial Wilt
Risk Potential*
Exclusion
(establishment precluded)
Low
High
*The Risk map is a combination of the Host and NAPPFAST maps. A Risk map depicts with a relative scale, the potential areas that are unsuitable or highly suitable for growth and establishment. It is possible to directly compare values between maps of the same type (e.g. Risk to Risk).
The U.S. Department of Agriculture's Animal and Plant Health Inspection Service collected the data displayed for internal Agency purposes only. These data may be used by others; however, they must be used for their original intended purposes.
Source: NASS 2007; USFS FIA; NAPPFAST
North America Albers Equal Area Conic (1983)
Data contact: Dan Borchert, USDA CPHST, Raleigh
Map created: March 2010 by USDA CPHST, Raleigh
1:20,000,000
0
250
500
Miles