Recovery Plan

for

Ralstonia solanacearum Race 3 Biovar 2

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

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Contents Page

Executive Summary		2
Contributors		4
I	Introduction	4
II.	Taxonomy – Subgroup Division	5
III.	Signs and Symptoms	9
IV.	Distribution, Spread and Risk Map	13
V.	Climate Change	14
VI.	Detection and Identification	15
VII.	USDA Pathogen Permits and Regulations	25
VIII.	Response	26
IX.	Economic Impact and Compensation	26
X.	Mitigation and Disease Management	27
XI.	Current Infrastructure, Needs and Experts	32
XII.	Research, Education and Extension Priorities	34
References		36
Web Resources		47
Appendix		48

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 *Pseudomonas solanacearum*. Historically, strains of *R. solanacearum* were classified into races, based loosely on host range, and then into 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 and typically has an extensive host range that includes important solanaceous crops. In the United States, *R. solanacearum* Race 1 is found in the southern states, where it causes bacterial wilt on a range of cultivated crops, such as pepper, tobacco, tomato, but rarely on potato. Bacterial wilt caused by this group has not spread to potato in other areas of the U.S. because the seed potato system is isolated from the potato production system. Furthermore, 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) originated in South American tropical highlands and can survive in temperate climates. In Europe, R3b2 has been responsible for several outbreaks of potato brown rot over the last several decades. The ability of R3b2 to survive cool temperature and its aggressiveness on potato make it a serious threat to American agriculture.

A revised classification system was proposed in 2005 based on DNA sequences and phylogenetic analysis. *R. solanacearum* was divided into four groups called phylotypes and the phylotypes were further subdivided into sequevars. R3b2 was placed in phylotype 2, sequevars 1 and 2.

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 was accidentally introduced into the U.S. on geranium cuttings produced in the highland tropics of Africa and Central America. During 1999 and 2000 R3b2 was found on some geranium cuttings imported into the U.S. In February 2003, the bacterium was again identified in geranium cuttings imported from a facility in Kenya. The 2003 introduction resulted in the organism being identified 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 identified 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 on solanaceous plants and geraniums. On potato, the disease 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. This bacterial strain is adapted to cooler temperatures and could be damaging to potato production regions of the US. While race 1 causes losses to tomato crops in the southern US, the economic impact R3b2 on potatoes and other host crops is unknown in temperate US climates affecting crops such as potatoes and other solanaceous crops. If introduced, R3b2 could affect a potato crop in the U.S. valued at $3.9 billion in 2015 with an export value of $1.4 billion (National Potato Council 2015) for a variety of potato products.

R3b2 is listed in the US as a “select agent”. This is 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 to register these agents with USDA, APHIS Plant Protection and Quarantine. Although only R3b2 is considered a select agent, all uncategorized strains of *R. solanacearum* are considered select agents until further classified by additional testing.

Management of brown rot 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 has limited efficacy. Additionally, detection of the pathogen can be difficult due to the occurrence of latent (symptomless) infection in potato tubers or in geranium cuttings. Only a few commercially acceptable and R3b2 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 to control 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 and soil disinfestation. 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) such as:
   a) Develop and/or screen additional chemical and biological control products.
   b) Using molecular and traditional techniques, develop resistant plant varieties that are commercially viable.
   c) Exclude the pathogen from potato seeds, geranium cuttings, and tomato transplants and develop vegetative plant material certification schemes.
   d) Study the effects of cover crops, crop rotation and mulches on pathogen dynamics and disease incidence.

2) Develop easy to use detection tools to reliably distinguish subgroups of *R. solanacearum*, especially R3b2, from other endemic strains of *R. solanacearum*.

3) Educate extension agents, growers and crop consultants in:
   a) sampling, monitoring and management of related diseases
   b) the utility of map-based tracking
   c) 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

The first reports of bacterial wilt appeared in the late 19th century. The pathogen was observed causing disease on tomato, tobacco, peanut and potato. Initially called "slime disease" it was found in subtropical regions including Asia, the Southeastern US, and South America. In 1896 Erwin F. Smith demonstrated that "slime disease" was caused by a bacterium which he named *Bacillus solanacearum*. Several years later, he transferred the bacterium into the genus *Pseudomonas* (Janse 2012; OEPP/EPPO 2004). Based on 16S rRNA sequences, fatty acid composition and phenotypic characteristics, Yabuuchi et al. (1992) transferred seven species of *Pseudomonas,* including *P. solanacearum* into the new genus *Burkholderia*. After additional work, Yabuuchi et al. (1995) transferred *Burkholderia solanacearum* to the new genus *Ralstonia.*

Ralstonia solanacearum is in the class Betaproteobacteria (Yabuuchi et al. 2005). It is an aerobic, gram negative rod with 1-4 polar flagella. It is oxidase positive and arginine dihydrolase negative. *R. solanacearum* produces intracellular poly-hydroxybutyrate crystals and this characteristic is sometimes used as a preliminary diagnostic (Denny and Hayward 2001).

The cold variant, which was later categorized as Race 3 biovar 2 (R3b2) was first described by Moraes (1947) in Portugal. This variant was found in other Mediterranean countries and in mountains in the tropics. Further investigation led researchers to conclude that R3b2 originated from potatoes in the Andes, and was possibly introduced into the Mediterranean area during World War II (Janse 2012).

USDA-APHIS designated *R. solanacearum* R3b2 as a select agent under the Agriculture Bioterrorism Act of 2002. A recent change in the select agent regulation has listed *R. solanacearum* as a select agent and all strains of *R. solanacearum* are considered select agents until further testing can determine that they are not R3b2 strains. In Europe, R3b3 is considered an A2 quarantine pest by the European and Mediterranean Plant Protection Organization (EPPO). In Canada (Canadian Food Inspection Agency 2014) and China it is classified as a quarantine pathogen (Li et al. 2014).

II. Taxonomy -Subgroup Divisions

Bacterial wilt is an important soilborne vascular disease that is distributed worldwide, affecting over 50 botanical families. The causal agent, *R. solanacearum*, is subdivided into five races, loosely based on host range and subsequently divided into five biovars, based on their ability to produce acid from a panel of carbohydrates (Denny 2006). There is no general correlation between races and biovars, except that 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, 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).

R. solanacearum is considered a species complex that includes *R. solanacearum*, *R. syzygii* and the blood disease bacterium (BDB). A revised, molecular based classification system was proposed in 2005 and some modifications have been made since that time. *R. solanacearum* strains were divided into four major genetic groups called phylotypes. These groups were assigned based on phylogenetic analysis of the 16S-23S intergenic linker sequence data, and confirmed by the hypersensitive response and sequence analysis of the pathogenicity gene *hrpB*, and the endoglucanase (*egl*) gene. The four phylotypes reflect the geographical origin and ancestral relationships between strains (Fegan and Prior 2005): Asia (phylotype I), the Americas (phylotype II), Africa and the surrounding islands (phylotype III), and Indonesia (phylotype IV, which includes *R. syzygii* and the BDB). The phylotype division is now well accepted by researchers working with *R. solanacearum*.

Prior and Fegan (2005) divided the phylotypes into sequevars based on a partial sequence of the *egl* gene. They define a sequevar as "a group of strains with a highly conserved sequence within the area of the gene sequenced." There are currently a total of 53 known sequevars within the four phylotypes. *R. solanacearum* race 3 biovar 2 (R3b2) belongs to phylotype IIB, sequevars 1 and 2 (IIB -1&2). The work by Fegan and Prior (2005) is summarized in Table 1.

Table 1: Summary of *R. solanacearum* classification scheme by Fegan and Prior (2005)

Phylotype and geographic origin	Sequevar	Race	Biovar	Host of origin
Phylotype I Asia and Ocenia	12-18	1 4 5	3 4 4 5 2T	many many ginger mulberry pepper, tomato
Phylotype IIB Americas	3,4 1,2	2 3	1 2 2T	musaceous potato potato, eggplant
Phylotype IIA Americas	5,7 6	1 2	1 1	many musaceous
Phylotype III Africa	19-23		1* 2T	many potato
Phylotype IV Indonesia and Asia	 11 8 10 9		1* 2 2T 2T & *R. syzygii* subsp. *celebesensis* 1 & R. *syzygii* subsp. *syzygii*	clove clove, potato potato musaceous clove

*Unrelated to biovar 1 in phylotype II
(From Denny 2006)

Wicker et al. (2012) subdivided the phylotypes into eight clades (Table 2). Clades were based on multilocus sequence analysis (MLSA) of nine single copy genes located either on the chromosome or megaplasmid.

Table 2: Clades of *Ralstonia solanacearum* from Wicker et al. (2012)

Phylotype	Clade	Sequevar	Disease	Geographic origin
Phylotype I	Clade 1	10,12,13,14,15,16,17,18, 31,34,44,45,46,47,48	BW*, Mulberry & BW	Central America & southeastern US
Phylotype IIA	Clade 2	6,24,35,36,38,39,41,50,52	Moko disease & BW	Austral Latin America
	Clade 3	7	BW	Northern Latin America and the Caribbean
Phylotype IIB	Clade 4	4, 51	Moko inducing and emerging strains isolated from *Anthurium* and *Helioconia*	Netherlands
	Clade 5	1, 2,3, 25,26,27	Potato brown rot & Moko disease	Central and West Africa
Phylotype III	Clade 6	19,20,21, 29, 42,43,44, 49	BW	Austral and eastern African, and Indian Ocean
Phyllotype IV	Clade 7	8, 10	Blood disease of Banana & BW	North and eastern Asia
	Clade 8	9	*R. syzgii*	Indonesia and northern Australia

*BW = bacterial wilt

Fegan and Prior (2005) define a species complex as "a cluster of closely related isolates whose individual members may represent more than one species." The idea of *R. solanacearum* as a species complex was initially proposed by Gillings and Fahy (1994) and then expanded upon by Taghavi et al. (1996). Remenant et al (2011) suggested that *R. solanacearum* should be divided into three species based on sequencing and DNA-DNA hybridization (Table 3). Safni et al (2014) disputed the names proposed by Remenant et al. (2011). They ran phylogenetic analysis on sequence data from the 16S-23S rRNA ITS gene sequences, 16S-23S rRNA intergenic spacer (ITS) region sequences and partial *egl* gene sequences. Based on these data, the *R. solanacearum* species complex of strains were reclassified into three genospecies, which correspond with the phylotype divisions. Only the current phylotype II strains would continue to be *R. solanacearum* (Table 3). Prior et al. (2016) confirmed the reclassification by Safni et al. (2014) using proteomic profiles and genomic sequence comparisons.

Table 3: Ralstonia classification scheme from Safni et al. (2014)

Phylotype	*Ralstonia* species (Remenant et al. 2011)	*Ralstonia* species (Safni et al. 2014)
I	*R. sequeirae*	*Ralstonia pseudosolanacearum*
II	*R. solanacearum*	*Ralstonia solanacearum*
III	*R. sequeirae*	*Ralstonia pseudosolanacearum*
IV	*R. haywardii* subspecies *celebensis*	*Ralstonia syzygii* subsp*. celebesensis*
	R. haywardii subspecies *solanacearum*	*Ralstonia syzygii* subsp*. indonesiensis*
	R. haywardii subspecies *syzygii*	*Ralstonia syzygii* subsp*. syzygii*

The origin of *R. solanacearum* is not clear; however, Hayward (1991) suggests that it predates the geological separation of the continents given that the bacterium has been found in virgin jungles in South America and Indonesia. However, race 3 biovar 2 strains are believed to have originated in the Andean highlands, although this near-clonal subgroup has been widely distributed throughout the world including tropical and some temperate regions, such as Europe and northern Asia.

Several race 1 strains of the pathogen are established in the southern United States, where they cause bacterial wilt of tomato and other crops, such as, tobacco, and rarely, pepper and potato. This race has not spread throughout the US, largely due to its inability to overwinter in northern environments and/or soils with low water holding capacity. Furthermore, the seed potato system is isolated from the production system. In contrast, R3b2 can survive in temperate climates including highland tropics, the UK, and the Netherlands (Elphinstone 2005), and usually in association with plant tissue (Milling et al. 2009). R3b2 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 geranium. The host range of this group is not as wide as race 1. However, it is of particular concern to the potato industry in the United States, and could extend the geographic range of *R. solanacearum* for other crops, such as tomato, within our borders.

The 8X draft sequence coverage of UW551 (R3b2 strain) genome was published and led to the identification of R3b2-specific genes (Gabriel et al. 2006). Draft genome sequences of three other R3b2 strains including IPO1609 from the Netherlands (Guidot et al. 2009), NCPPB909 from Egypt and CFIA 906 from India (Yuan et al. 2015) were also determined. Recently, the first complete genome sequence of UY031, the R3b2 strain isolated in Uruguay, has been published. The R3b2 genome information will facilitate future development of molecular diagnostic assays for specific detection and differentiation of R3b2.

III. 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 (Fig 1A). 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. 1B). Initially the vascular ring appears yellow to light brown, but as the infection progresses the ring will become browner (Champoiseau et al. 2009).

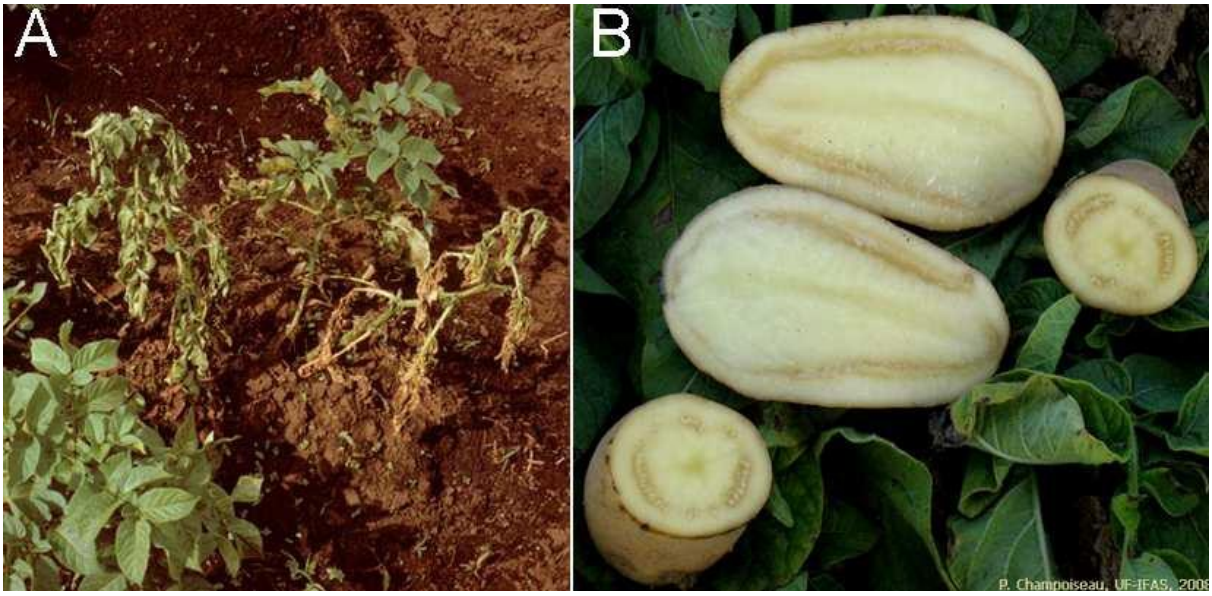


Figure 1. Symptoms of brown rot caused by *R. solanacearum* race 3 biovar 2 on potato. Photo credits: D. Thurston, Cornell University (A) and P. Champoiseau, University of Florida -IFAS (B).

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

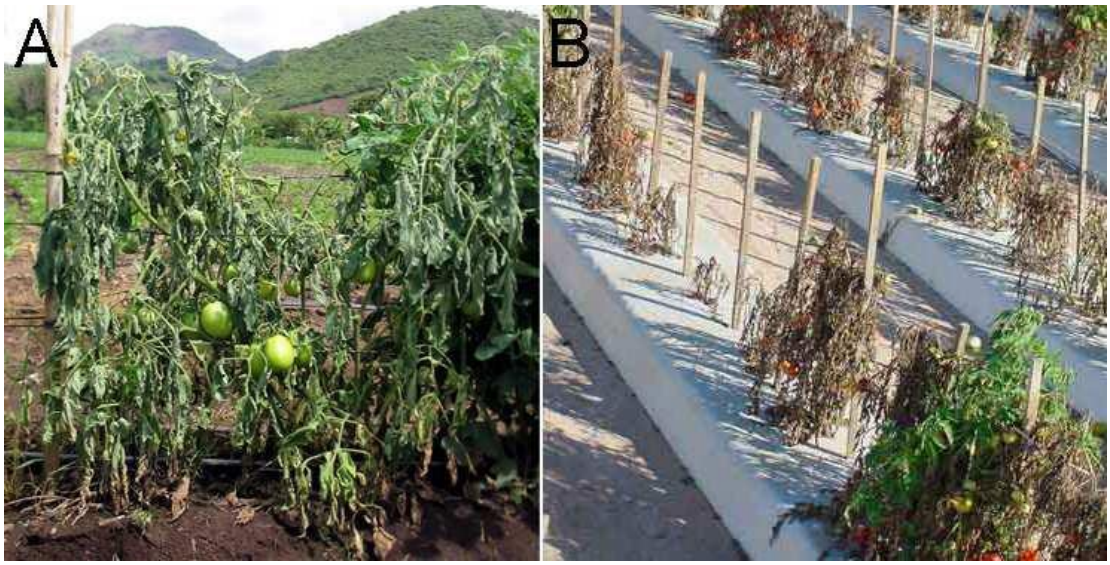


Figure 2. Symptoms of bacterial wilt of tomato caused by *R. solanacearum* in Guatemala (A) and Florida (B). Photo credits: C. Allen, University of Wisconsin (A) and T. M. Momol, University of Florida-IFAS (B).

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 bacterial wilt, also called southern wilt, of geranium is the upward curling of leaf margins. 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 hortorum* 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) (Champoiseau et al. 2009).

2. Signs of the pathogen:

Bacterial streaming is a common diagnostic sign of *R. solanacearum*. When freshly cut stem sections from infected plants are placed in water, threads of a viscous white slime often can be observed 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 the 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 in the early stage of 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 and due to the dynamics of plant-host interactions, 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 the spread of *R. solanacearum* and in the epidemiology of this disease. It was shown that latently infected geranium cuttings were responsible for introduction of R3b2 to the U.S. in 2003 and 2004. Janse et al. (2004) demonstrated that both *Petunia* and *Calibrachoa* can also harbor latent infections. Latent infection can also be present in resistant or tolerant crop varieties (Lebeau et al. 2011). Sharma et al. (2016) reported that latently infected seed potato fields were identified in the Oromia and SNNP regions of Ethiopia. Latently infected seed potatoes are the major source of bacterial wilt outbreaks in Ethiopia (Parker et al. 2016).

In their survey of commercial fields in Thailand, Dittapongpitch and Surat (2003) found latent infection in tomatoes and peppers growing in a field that had previously had high losses due to the disease. Some varieties of plants once considered to be resistant to *R. solanacearum* were actually tolerant, as they could harbor the bacteria without showing symptoms (Huet 2014).

Of great concern is latent infection of *R. solanacearum* in weeds, found in previously infested fields and along waterways. *Solanum dulcamara* or bittersweet is a weed that commonly grows along waterways and in other wet areas. Although native to Europe, it is now found in many parts of the world, including the US (Waggy 2009). Latent infection in *S. dulcamara* has been documented in Norway, the Netherlands and England (Janse 1996). Latent infections have also been found in a variety of weeds worldwide. Some examples are listed in Table 4.

Table 4: List of selected weeds where latent infection by *R. solanacearum* has been documented

Weed	Country	Location	Citation
Amaranthus spp.	Thailand	potato field	Dittapongpitch and Surat (2003)
	Uganda	potato field	Tusiime et al. (1998)
Bidens pilosa	Uganda	potato field	Tusiime et al. (1998)
Dopatrium sp.	Nepal	rice field	Pradhanang and Momol (2001)
Monochoria vaginalis	Nepal	rice field	Pradhanang and Momol (2001)
Physalis minima	Thailand	potato field	Dittapongpitch and Surat (2003)
Polygonium napalense	Uganda	potato field	Tusiime et al. (1998)
Euphorbia hirta	Thailand	potato field	Dittapongpitch and Surat (2003)
Rumex abyssinicus	Thailand	potato field	Dittapongpitch and Surat (2003)
	Uganda	potato field	Tusiime et al. (1998)
	France	river basin	Janse (1996)
Solanum dulcamara	England	river basin	Janse (1996)
	Netherlands	river basin	Janse (1996)
	Netherlands	river basin	Wenneker et al. (1999)
	Norway	river basin	Janse (1996)
Spergula arvensis	Thailand	potato field	Dittapongpitch and Surat (2003)
	Uganda	potato field	Tusiime et al. (1998)
Urtica dioica	Netherlands	river basin	Wenneker et al. (1999)

4. Look-alike symptoms:

Symptoms caused by *R. solanacearum* are similar to those caused by other pathogens, such as *Fusarium oxysporum* f. sp. *lycopersici* causing *Fusarium* wilt on tomato and *V. albo-atrum* and *V. dahlia* causing *Verticillium* wilt on potato. Abiotic stresses can produce symptoms that can be confused with bacterial wilt. These include mechanical root damage, drought, or nutrient deficiency. Therefore symptoms alone should not be used for diagnosis. For this reason, caution is needed in determining if a symptomatic plant is actually infected with *R. solanacearum*. This is especially true for bacterial wilt caused by R3b2, since similar symptoms in the same or different plants may be caused by race 1 of the pathogen, which is endemic in the southern US. Furthermore, the current commercial ELISA and immunostrip tests will react with both R3b2 and the endemic race 1 strains of *R. solanacearum*.

IV. Distribution Spread and Risk Map

R. solanacearum is primarily a soilborne and waterborne pathogen. It can be and is disseminated by infested soil, contaminated irrigation water, surface water, equipment, or personnel, as well as infected plant material, including latently infected 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* normally enters host plants from soil through wounds in the roots formed by lateral root emergence or caused by agriculture process or soilborne organisms (e.g. the root-knot nematode). The bacterium can also enter plants by way of stem injuries caused by 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 entered the U.S. several times 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 found 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, however, the bacterium was again identified in geranium cuttings imported from a facility in Kenya. The outbreak was caused by a breach in sanitation in Kenya that led to the contamination of 7 stock plants. As a result, R3b2 was detected in 127 individual greenhouses in 27 states in the U.S. 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 USDA APHIS. It was created 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 likelihood of pest growth and survival. A value of 10 indicates high density of susceptible hosts and a likelihood of pest growth and survival.

R. solanacearum occurs worldwide. Although in the past R3b2 has been found in the U.S. from imported geranium cuttings, currently there are no active areas where the pathogen is known to be present. Other strains of *R. solanacearum* such as the race 1 strains, are found in the U.S. The EEPO Global Database maintains an on-line *R. solanacearum* race 3 distribution map which can be found at: https://gd.eppo.int/taxon/PSDMS3/distribution (accessed 02/12/2017). The site also contains incident reports, surveys and other related information.

V. Climate Change

Luck et al. (2011) observed that the significance of climate on plant diseases has been known for over 2000 years, dating back to ancient Greece. However, increase in temperature and carbon dioxide concentrations has accelerated during the past 100 years (Gautam et al. 2013). It has been predicted that worldwide temperatures will continue to increase due to an increasing concentration of greenhouse gases in the atmosphere. The atmospheric concentration of carbon dioxide, methane and nitrous oxide, the so called greenhouse gases, has risen significantly since 2000 (Yanez-Lopez et al. 2012). Climate change driven phenomena such as warmer winter temperatures, elevated humidity, cyclones, hurricanes drought, and increased CO_2 are the factors likely to influence plant diseases (Luck et al. 2011).

Schaad (2008) observed that in recent years several bacteria, including *R. solanacearum* have become significant worldwide pathogens. He suggests that one possible explanation could be global warming. These bacteria (*Acidovorax, Burkholderia, Ralstonia*) grow well at higher temperatures compared to many other plant pathogenic bacteria. The projected weather conditions associated with climate change, such as heat waves, long periods of rain and severe storms and hurricanes will favor the occurrence of diseases caused by these bacteria. Temperature is viewed as one of the most important factors affecting the distribution of many plant pathogenic bacteria. If, as a result of climate change, temperatures continue to rise worldwide, then the diseases caused by heat-loving plant pathogenic bacteria should be expected to increase (Kudela 2009).

Janse (1996) noted that outbreaks of brown rot (caused by R3b2) are increasingly found in Western Europe. Two exceptionally warm summers in 1994 and 1995 might have facilitated the outbreak. Schaad (2008) noted that 1995 was the warmest year in Europe since record keeping began in 1856. To date, 2014 is currently the warmest year on record for Europe (http://www.noaanews.noaa.gov/stories2015/071615-international-report-confirms-2014-was-earths-warmest-year-on-record.html, accessed 2/12/2017), though no reports were available documenting the severity of bacterial wilt in Europe for that year.

Haverkort and Verhagen (2008) stated that weather conditions associated with climate change such as irregular rainfall, higher intensity rainfall, flooding and higher temperatures will increase the risks of bacterial diseases in potato, including bacterial wilt. Castillo and Plata (2016) looked at the spread of brown rot in Bolivia and found that *R. solanacearum* was found at higher altitudes than previously recorded. They suggest that this could be attributed to climate change since minimum temperatures in the Andes are increasing. Climate simulation models show an increase in winter precipitation at higher latitudes (Chakraborty et al. 2000). Projected increases in precipitation and more extreme events such as floods, heavy downpours, and storms could also facilitate the survival of *R. solanacearum* in the Bolivian highlands. They further projected that these factors could increase the spread of the pathogen into areas where previously it did not survive. They also noted that the spread of the pathogen might also be attributed to the increase of latently infected tubers in areas that were previously pathogen free (Castillo and Plata 2016).

Huerta et al. (2015) studied the interaction between tropical strains of *R. solanacearum* and the race 3 biovar 2 strain at high (28°C) and low (24/19°C) temperatures. They note that although R3b2 can be found in contaminated surface water and imported plant material in the tropical lowlands, it has not been isolated from wilting plants. Individually, all strains grew well at both temperatures. When strain competition was tested *in vitro* and *in planta* using R3b2 and two tropical lowland isolates, both tropical strains outcompeted R3b2 at warmer temperatures. R3b2 outcompeted the other two strains at cooler temperatures. Further tests demonstrated that the two tropical strains produced a bacteriocin like compound that inhibited the growth of R3bv2. However, R3b2 was able to outcompete the tropical lowland strains at cooler temperatures without the benefit of bacteriocins.

The geographical distribution of plant pathogens and their hosts will likely be altered as a result of climate change (Kudela 2009). There has been limited research on climate change's effects on pathogens of field crops. However, results show that severity of disease caused by pathogens such as *R. solanacearum* may increase, decrease or remain unchanged. The change in disease severity will depend on the region and/or weather variables used in the model (Luck 2011).

Although climate change may act as a driving force in altering the geographic range of *R. solanacearum* and plant diseases in general, it will clearly not be the only factor at play. Research by Heurta et al. (2015) demonstrates that other non-climate factors could also be important. Extrapolating from their study, factors such as the microbial community, microflora, microfauna, nematodes, and soil type may also play a role in the redistribution of plant diseases.

VI. 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: https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs Detailed instructions for submitting samples are available in the document located at: https://www.aphis.usda.gov/plant_health/plant_pest_info/ralstonia/downloads/guidance-domestic-sample-handling.pdf (accessed 02/12/2017).

1. Preliminary diagnostic screening tests:

a. Bacterial streaming.

Clouds of bacterial cells usually appear within 20 minutes when severed petioles or stems of the suspected plants are placed in tubes of sterile water as described above (Fig. 4). Note that bacterial streaming 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. The streaming test is a valuable diagnostic tool for quick detection of bacterial wilt or brown rot in the field. Although this test demonstrates the presence of a bacterial plant pathogen it is not specific to R3b2 or even to the genus *Ralstonia*.

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. Currently there are several available test kits worldwide (Table 5).

Table 5: Serological test kits for *R. solanacearum*

Product name	Part Number	Lower detection limit	**Manufacturer**
Rs ImmunoStrip Test	ISK 33900	10^5 CFUs	Agdia, Inc., USA
POCKET DIAGNOSTIC® *Ralstonia solanacearum* ,	PD51119	10^5 CFUs	Pocket Diagnostics, Abingdon Health , York**,** UK.
Ralstonia solanacearum EXPRESS™ *	1091-13	10^6 CFUs	Neogen Europe Ltd., Scotland, UK

*Test not listed in Floyd (2008). SPOTCHECK LF™, which was validated as indicated by Floyd (2008) is no longer available from this vendor.

Serological screening tests are generally quick, inexpensive, reliable, and require minimum equipment. The minimum detection level (sensitivity) of these tests is 10^5-10^6 colony forming units (cfu). However, they cannot be used to identify the race or biovar, and can only detect high populations of the pathogen. Furthermore, the current commercial ELISA or immunostrip tests will react with both R3b2 and endemic race 1. It is also important to understand that serological methods do not differentiate between living and dead cells. This becomes an issue with regard to decisions relating to quarantine issues and destroying contaminated shipments (Denny 2006).

c. Cellular analysis and notification of antigen risk and yield (CANARY) technology

The CANARY system is a modified cell based system using b lymphocytes. Lymphocytes are modified to express a single antibody, specific to the organism of interest. Aequorin, a luminescent protein obtained from jellyfish is also expressed in the cell and becomes activated to emit light when the specified antigen is detected. The advantages of CANARY are that it has a relatively low detection threshold and low rate of false positives (Materials and Manufacturing Processes for Advanced Sensors 2004). Systems are commercially available and manufactured by Pathsensors, Inc. (Baltimore, MD 21202)

The assay is simple and quick. Lymphocytes and then the sample are added to a microfuge tube and centrifuged to mix. The reaction is read in a luminometer. The entire test can be completed in a few minutes. Tests have shown the minimum detection limit for *R. solanacearum* using the CANARY system is 10^3 CFUs - which is comparable to qPCR. This system is also able to detect *R. solanacearum* from plant tissue that is in poor condition and that would typically yield a false negative reaction using other diagnostic tests (Levy et al. 2013).

Since February 2014, the USDA APHIS has been evaluating the CANARY technology at the inspection stations in Atlanta, GA and Linden, NJ. Currently the system is being used to test cuttings of *Pelargonium* spp. (geranium) that did not originate in USDA approved facilities. Any samples testing positive for *R. solanacearum* must be verified as being R3b2 by other methods (http://content.govdelivery.com/accounts/USDAAPHIS/bulletins/a2fe46, accessed 02/12/2017)

d. ***Automated methods of identification***

i. The Sherlock™ Microbial Identification System and fatty acid analysis

The Sherlock™ Microbial Identification System developed by MIDI, Inc. (Newark, DE) can identify bacteria to species based on their fatty acid profiles. The RTSBA6 library version 6.21 contains three species of *Ralstonia*, including *eutropha*, *pickettii*, and *solacacearum*. When grown under their standard conditions (Sasser 2006) the fatty acid profile for *R. solanacearum* is listed in Table 6.

Table 6: Fatty Acid Profile for *R. solanacearum* from MIS database

Fatty Acid Name	% of profile
Summed Feature 3 (16:1 w6/7c)	26.46
16:00	26.34
Summed Feature 8 (18:1 w6/7c)	18.77
Summed Feature 2 (14:0 3OH and its breakdown	7.45
17:0 cyclo	5.16
18:1 2OH	4.87
16:1 2OH	4.57
14:00	4.39
16:0 2OH	0.89

There have been several papers demonstrating the use of fatty acid profiles for identification of *R. solanacearum*. Salete de Melo et al. (1999), Khakvar et al. (2009) and Behiry et al. (2015) all conducted research on using fatty acid profiles to distinguish among *R. solanacearum* strains.

Although there was some preliminary evidence suggesting that fatty acid profiles might be able to separate out strains by host or by geographic origin, there was no evidence that the profiles could be used to distinguish biovars or sequevars. Janse (1991) was able to distinguish races and virulent from avirulent strains using fatty acid analysis. It should be noted that each study grew their strains under different conditions and used different extraction methods. Therefore, the results are not comparable. More work needs to be done using a larger number and greater diversity of strains, under standard conditions to determine the usefulness of fatty acid profiling for distinguishing subgroups of *R. solanacearum.*

ii. The Biolog System and substrate utilization

The GEN III system (version 2.8) from Biolog (Hayward, CA) identifies bacteria based on a 96-test profile of carbon source utilization and chemical sensitivity. The database contains four species of *Ralstonia*, including *R. insidiosa, R. mannitolilytica, R. pickettii, and R. solanacearum*. The Biolog plate includes seven of the eight of the carbohydrates used in the *R. solanacearum* biovar test. The seven carbohydrates and well locations are: glucose (C1), maltose (A3), trehalose (A4), cellobiose (A5), lactose (B2), sorbitol (D1), and mannitol (D2). The plate does not include dulcitol. Although this system has not been validated for identification of biovars, it can be useful as a preliminary screen to rule out biovar 2.

e. *Bacteriophages*

Kutin et al. (2009) developed a protocol using bacteriophages combined with qPCR to detect *R. solanacearum* in plant tissue. The assay was very sensitive in detecting both active and latent infections at concentrations as low as 2.7×10^2 CFUs/g of plant tissue. This method is also useful because it eliminates the problem of PCR inhibitors in plant tissue and only detects live cells.

In his review of bacteriophages of *R. solanacearum*, Yamada (2012) discusses the potential use of bacteriophages for detection of *R. solanacearum* in plants and soil. The filamentous phages RSS1 and RSM1 were identified as possibly being useful for bacterial and strain identification. However, there are currently no validated methods for using bacteriophages to identify *R. solanacearum* to the biovar level.

2. Isolation, culture and identification:

a. Water, plant and soil sampling.

With experience, *R. solanacearum* can be isolated and cultured from diseased tissue and infested soil and water samples. Optimized sampling protocols from USDA-APHIS (Floyd, 2008) 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 (Stevens and van Elsas, 2010, Wenneker et al. 1999).

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

The bacterium may be cultured from diseased tissue, soil and water samples in a diagnostic laboratory by streaking from soil suspensions, diseased tissue suspensions, or water samples onto the SMSA medium (Elphinstone et al. 1996). The SMSA medium contains antibiotics and fungicides that will inhibit or reduce growth of competing saprophytic bacteria and 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. This could create confusion, resulting in false positives, if only the semi-selective medium is relied upon for detection. This test is not diagnostic for race or biovar.

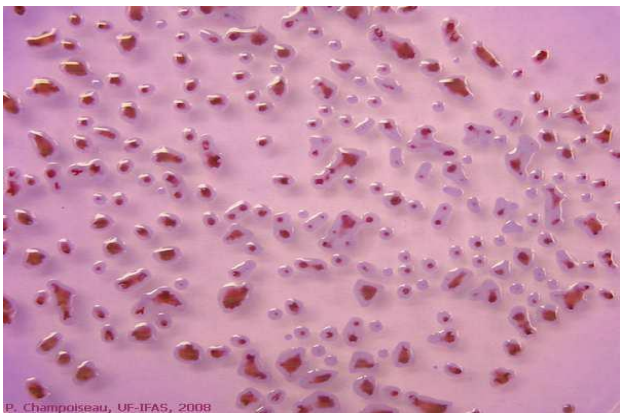


Figure 6. Virulent colonies of *R. solanacearum* on modified SMSA medium after 4 days. (P. Champoisea, UF-IFAS)

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 bacterium (Denny and Hayward, 2001). A tetrazolium chloride medium (TZC) can help to differentiate virulent colonies from non-virulent ones. While virulent colonies are white or cream-colored, irregularly shaped, highly fluidal, and opaque, non-virulent types 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 36 hrs at 28°C 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 bacterial translocation 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

i. DNA extraction

There are many DNA extraction kits commercially available. Floyd (2008) evaluated several methods for DNA extraction and found that two products, performed the best and were the most reliable. These are the Qiagen DNeasy Plant Mini Kit (part # 69104 or 69106) and QIAamp DNA Mini Kit (part # 51304 or 51306), both manufactured by Qiagen (Germantown, MD).

Stulberg et al. (2015) and Remenant et al. (2010) successfully utilized Qiagen's DNeasy Blood and Tissue Kit (part # 69504 or 69506) to extract genomic DNA from *R. solanacearum*. Albuquerque et al. (2014) used the AxyPrep Bacterial Genomic DNA MiniPrepKit (Axygen Biosciences, Union City). Baharuddin et al. (2014) and Guidot et al. (2007) each used different versions of a phenol:choloroform extraction protocol. Recently, Stulberg and Huang (2015) developed an extraction buffer for a quick, inexpensive and easy DNA extraction from infected plants including geranium for detection of *R. solanacearum* by qPCR.

Other commercial kits for bacterial DNA extraction include the Wizard® Genomic DNA Purification Kit from Promega (Madison, WI), UltraClean® Microbial DNA Isolation Kit from Mo-Bio (Carlsbad, CA), and PureLink™ Microbiome DNA Purification Kit from Invitrogen (Carlsbad, CA). Extraction methods that use phenol and chloroform, as well as other extraction protocols can be found in publications on molecular biology techniques such as those by Green and Sambrook (2012) and Ausebel et al. (2003). Choice of a kit and/or method should be based on whether DNA is being extracted from cultures, infected plant material, soil, or water and the intended use of the extracted DNA.

ii. Flinders Technology Associates (FTA) cards

FTA cards are manufactured by Whatman Technology, a brand of GE Healthcare (Marlborough, MA). They contain a proprietary matrix, which lyses cells while preserving and protecting the DNA. Grund et al. (2010) first documented that DNA from *R. solanacearum* could be stored on FTA cards and later amplified by PCR. Tran et al. (2016) observed that cells were killed within 60 min of being applied to a Whatman FTA (R) card. When PCR was run using extracts from a FTA card, the sensitivity level was approximately 10^4 CFUs – a level similar to that found in latently infected plants.

Chandrashekara et al. (2012) observed sensitivity levels as low as 29 CFU/ml. In this study, they collected, stored and amplified DNA from *R. solanacearum* infected solanaceous plants. Results using the FTA cards compared favorably to results from DNA extracted directly from plant sap. FTA cards will also bind PCR inhibitors present in plant tissue, such as in geraniums. However, inhibitors can also be eliminated prior to running PCR with additional treatment steps in the processing (Tran 2016). Another benefit is that samples on FTA cards can be stored at room temperature and no refrigeration is needed. Chandrashekara et al. (2012) found that when stored at room temperature the DNA was stable for at least 6 months.

Jenkins et al. (2014) demonstrated that FTA cards may provide a secure method for collecting *R. solanacearum* DNA from suspect plants in the field, particularly in remote locations, and transferring them to a lab for testing. With the increasing occurrence of bacterial wilt, FTA cards could become a valuable aid in the process of sampling and diagnosis.

iii. PCR and qPCR.

Race determination using PCR 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 qPCR assays that use phylotype/sequevar or biovar-specific primers can be used for identification of IIB-1&2 or biovar 2 strains of *R. solanacearum* (Fegan and Prior 2005; Stulberg and Huan, 2015; Stulberg et al. 2015 & 2016; Weller et al. 2000). QPCR 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) (Opina et al. 1997) and a 357-bp fragment that is found only in R3b2 strains (primer pair 630/631) (Fegan et al. 1998). Strains of *R. solanacearum* can be sub-classified into phylotypes with a single multiplex PCR reaction. Sequevar designation requires PCR amplification and sequencing and phylogenetic analysis of the conserved endoglucanase (*egl*) gene (Fegan and Prior 2005).

Stulberg et al. (2015) developed a multiplex PCR assay targeting non-phage genome sequences that identifies *R. solanacearum* species complex strains, differentiates the select agent R3b2 (IIB-1&2) strains from those that are not, and eliminates false negatives due to PCR inhibition or unsuccessful DNA extractions in one reaction (Table 6). The *R. solanacearum* species complex-specific primers target the predicted glycosyl transferase domain and the IIB-1&2-specific primers target the predicted ferric siderophore receptor. The lowest detection limit is 200 CFUs/PCR reaction.

Table 6: Primers used for the detection and identification of *R. solanacearum* at the species complex and R3b2 levels

Primer	Primer sequences	size (bp)	Target	Specific
759/760-	GTCGCCGTCAACTCACTTTCC- GTCGCCGTCAGCAATGCGGAATCG Opina et al. (1997)	282		*R. solanacearum* species complex strains
630/631	5' ATACAGAATTCGACCGGCACG 3' 5 AATCACATGCAATTCGCCTACG 3' Fegan et al. (1998)	357		R3b2 strains
RsSC-F RsSC-R	CCGAGCGCATATCGTTCACAC TTTGGCGTTC CGGTCGGAG Stulberg et al. (2015)	296	Predicted glycosyl transferase	*R. solanacearum* species complex strains
RsSA-F RsSA-R	CAACGATGCCTG GAAACTGACC TGGTCCGGGTTCAGGTAAATGTCAC Stulberg et al. (2015)	132	Predicted ferric siderophore receptor	IIB-1&2 (R3b2) strains of *R. solanacearum*

Stulberg and Huang (2016) identified specific single nucleotide polymorphisms (SNPs) for *Ralstonia* genospecies and for sequevars 1 and 2 by comparing their *egl* genes. They also designed primers (Table 7) to amplify a 526-bp *egl* fragment from *R. solanacearum* for easy sequencing of the amplicon, and to facilitate direct and specific amplification of *egl* from *R. solanacearum*-infected plants. This eliminates the need for bacterial isolation from infected plant material to obtain pure cultures. They then wrote a user friendly *R. solanacearum* typing program that will place a partial *egl* sequence into one of the three newly proposed *Ralstonia* genospecies and also determine if it is the highly regulated sequevars 1 or 2.

TaqMan-based real-time qPCR is commonly used in federal and state diagnostic laboratories due to its speed and sensitivity. Recently, Stulberg and Huang (2015) have developed a multiplex qPCR assay that identifies and confirms IIB-1&2 (R3b2) strains, and controls for false negatives. One of their IIB-1&2-specific qPCR primers and probe sets has been applied in a portable POCKIT™ system which has the potential to facilitate R3b2 detection at the ports of entry and in field settings (Di et al. 2016). Stulberg et al. (2016) have also developed new primers and probe sets, and systematically validated and compared them with the previously published ones for reliable detection of *R. solanacearum* at both the species complex and R3b2 levels by qPCR.

Table 7: Primers for amplification and sequencing of a partial endoglucanase gene for determination of genospecies and sequevars 1 & 2 of *R. solanacearum* (Stulberg and Huang, 2016)

Primer designation	Primers 5'- 3'	# of base pairs
	Amplification primers	
MJS-amp-egl-F	CGGACACGGACACCACGACTCTGAA	526
MJS-amp-egl-R	TAGCGCGCATAGTTGTGCGGATCGAGC	
	Sequencing primers	
MJS-seq-egl-F	ACACGGACACCACGACTCTG	413
MJS-seq-egl-R	GCATAGTTGTGCGGATCGAGC	

iv. Loop mediated amplification (LAMP)

LAMP was developed by Notomi et al. (2000). The protocol uses *bst* DNA polymerase large fragment and the reaction mixture is incubated at approximately 60-65°C for one hour. This technique has been successfully used for detection of R3b2 and offers some advantages over conventional PCR. Using this method, DNA can be amplified under isothermal conditions. Therefore, an expensive thermocycler is not required.

Kubota et al. (2011a) developed a non-instrumental system for running LAMP reactions under low-tech conditions. By combining calcium oxide powder with water, a temperature of 63°C can be obtained and maintained in an insulated container for one hour. A precipitate is formed by pyrophosphate, which is a by-product of DNA strand synthesis. A positive reaction is indicated by the white pyrophosphate precipitate. This provides a visual confirmation that the DNA amplification was successful, without running a gel.

Lenarcic et al. (2014) also worked with LAMP and found that it worked best when the *egl* gene was targeted. However, Tran et al. (2016) obtained 5-25% false negative results when testing infected geranium plants using LAMP. Kubota et al. (2011b) and Tran et al. (2016) obtained false positives due to cross contamination. As LAMP evolves, this technique has the potential to become a powerful and low tech DNA based tool for the detection of R3b2 particularly in remote areas lacking infrastructure.

b. Carbohydrate utilization

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 that can be used routinely in combination with other methods in diagnostic labs (Fig.7).

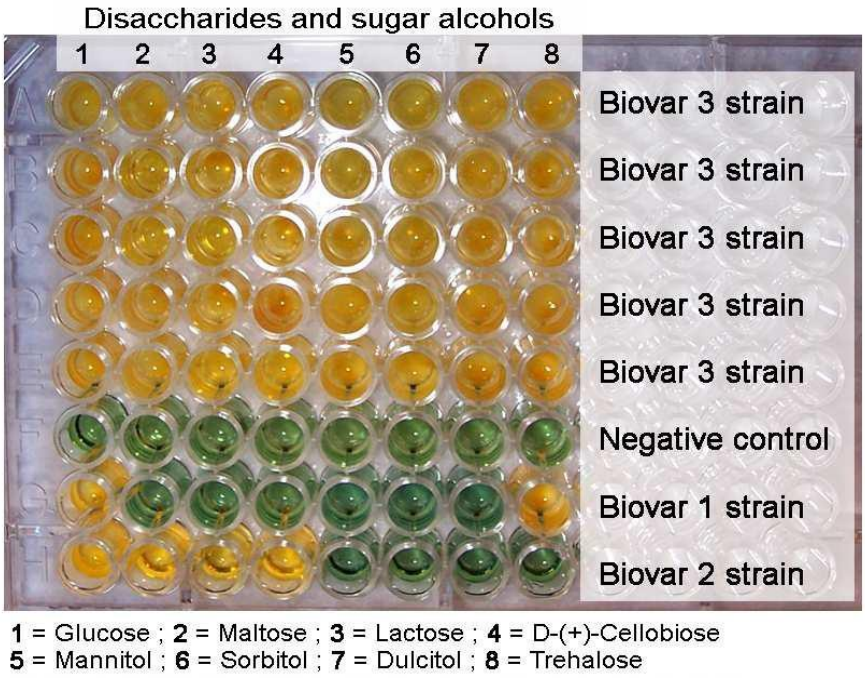
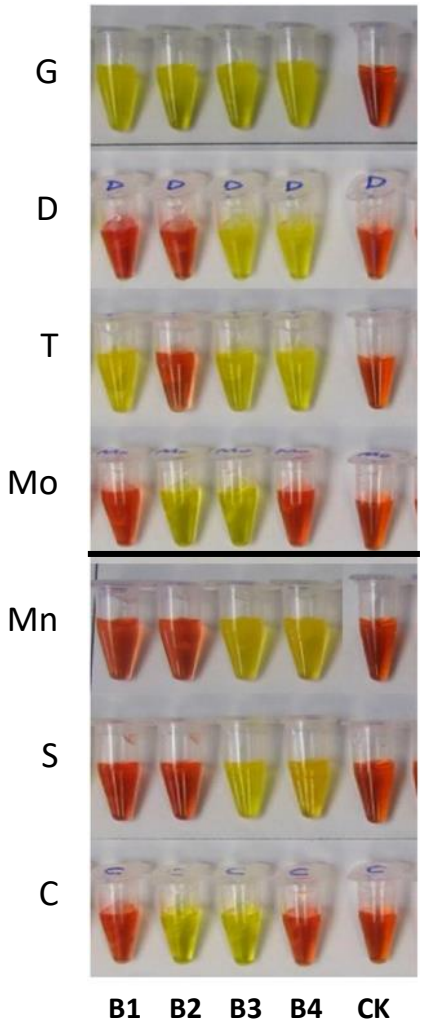


Figure 7. A microtiter plate showing the results of a biovar test with seven strains of *R. solanacearum*. Photo credit: E. N. Twieg, USDA-APHIS-PPQ-CPHST- National Plant Germplasm and Biotechnology Laboratory, Beltsville, MD.



Huang et al. (2012) modified the original biovar carbohydrate utilization test to increase the speed and efficiency (Fig. 8). They used 150 µl of medium in 200 µl PCR strip tubes instead of 3 ml of medium in 15 ml tubes, saving money and space. They also conducted the test at 32°C instead of 24-28°C and used phenol red as a pH indicator instead of bromthylmol blue. Phenol red changes color at a higher pH, reducing the time needed for the test to complete from 8 days to 4 days. In addition, they demonstrated that the number of carbohydrates needed for the test could be reduced from seven to four.

Biovars 1, 2, 3 and 4 can be differentiated in 3 days even if only the four carbohydrates above the black line were used.

Figure 8. Indicator changes in improved biovar tests four days after inoculation. G, glucose; D, dulcitol; T, trehalose; Mo, maltose; Mn, mannitol; S, sorbitol; C, D (+) cellobiose. B1, biovar 1 strain Rs124; B2, biovar 2 strain UW552; B3, biovar 3 strain Pss106, B4, biovar 4 strain Pss262; and ck, CPG broth (From Huang et al. 2012).

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 qPCR). 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.

VII. 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 2008).

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: https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information (accessed 02/12/2017) 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. Any bacterium identified as *R. solanacearum* is considered a select agent until the race and biovar have been determined. Guidelines for submitting samples can be found at the following website: https://www.aphis.usda.gov/plant_health/plant_pest_info/ralstonia/downloads/guidance-domestic-sample-handling.pdf (accessed 02/12/2017).

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 the agent or transfer it 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 Federal Select Agent web site: http://www.selectagents.gov/index.html (accessed 02/12/2017). The APHIS Select Agent Program can be contacted by phone at 301-851-3300 (select option 3) or e-mail: AgSAS@aphis.usda.gov

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.

VIII. 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 USDA, 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 2008).

IX. 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 2008). 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.

Corral, et al. (2013) report that crop insurance will cover losses from plant diseases and give an excellent discussion on the subject. Additional information can also be obtained on the USDA Risk Management Agency's web site (http://www.rma.usda.gov/, accessed 02/12/2017).

X. 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 a 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.

Lebeau et al. (2006) tested multiple strains of *R. solanacearum* against 30 resistant accessions of tomato, eggplant and pepper. Only eggplant and pepper showed resistance to R3b2. However even in the accessions with a low percentage of wilt, stem colonization of *R. solanacearum* ranged from 10 to 93%. Three cultivars of eggplant resistant to bacterial wilt have been used in India (Gopalakrishnan et al. 2005).

Another study evaluated resistance to bacterial wilt in 82 accessions of *Solanum* (formerly *Lycopersicon*) spp. Only two accessions showed partial resistance. These were the tomato cultivar Hawaii 7996 and *Solanum pimpinellifolium* accession # 63280 with 52% and 63 % wilt respectively (Carmielle et al. 2006; Huet 2014).

Seven genotypes from two wild Andean potato species were found to have high levels of resistance to wilt and tuber infection, which provide new resistance sources for developing commercial resistant potato cultivars (CIP 2004). *Solanum phureja*, a relative of the cultivated potato has shown resistance to bacterial wilt. It has been used in breeding programs for several decades. However, the resistance is only effective at cool temperatures or high altitudes (Patil et al. 2012). When Aliye et al. (2015) screened 55 potato cultivars for resistance to bacterial wilt, 28 showed resistance in greenhouse trials. However, when these 28 resistant cultivars were tested in the field, only two showed any resistance. This study highlights the significance of cultural and environmental conditions on the expression of resistance.

The difficulty of developing resistant plants in an interdisciplinary and interinstitutional 30 year program, based in Brazil is described by Lopez et al. (2016). Approximately 1% of the plants will be selected for disease resistance when screening plants from true seed for disease resistance using greenhouse inoculations. Accessions must then be tested in the field. Through this program, several clones were selected for stable resistance to bacterial wilt under field conditions. They are currently being used in a breeding program to also select for good quality tubers.

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 a 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. Chemicals tested for the control of bacterial wilt include:

- Actigard (e.g., acibenzolar-*S*-methyl, ASM) (Anith et al. 2004; Pradhanang et al. 2005; Abo-Elyousr et al. 2012)
- BABA (DL-3-aminobutyric acid) (Hassan and Abo-Elyousr 2013)
- Phosphorous acid (Norman et al. 2006; Ji et al. 2007)
- SBP (stable bleaching powder, calcium hypochlorite) and a urea-lime mix (Dhital et al. 1997)

In summary, it has been demonstrated that these chemicals have both greenhouse and to a lesser extent field efficacy. However, they have not been validated and tested on a larger scale against R3b2.

Soil fumigation with vapam, methyl bromide, or chloropicrin is thought to be of limited efficacy and utility (Yamada 2012). However, in field trials Mao et al. (2014) obtained good control of *R.*

solanacearum infection on ginger with methyl bromide and chloropicrin. Although the *R. solanacearum* isolate infecting ginger is race 4 biovar 4, this study is still applicable for control of R3b2 strains. It should be noted that the use of methyl bromide has been phased out as per the Montreal Amendment in 1997 of The Montreal Protocol on Substances that Deplete the Ozone Layer in 1987. The phase out was effective in 2005 for developed countries and in 2015 for developing countries (https://www.epa.gov/ozone-layer-protection/international-treaties-and-cooperation, accessed 02/12/2017).

During the past decade, the plant-derived essential oil, thymol, has been evaluated as a soil fumigant to control *R. solanacearum.* It was found to effectively reduce bacterial wilt incidence on tomato when used as pre-plant soil fumigation (Ji et al. 2005). Popoola et al. (2012), Pradhanang et al. (2001) and Hong et al. (2011) all observed some disease control when soil was treated with thymol. In addition, several other plant essential oils have been tested against *R. solanacearum* under greenhouse conditions Excellent control was demonstrated for bacterial wilt of tomato by palmarosa oil and lemongrass oil (Pradhanang et al. 2003), and bacterial wilt of tomato and geranium by clove oil (Huang and Lakshman 2010). Work by Hosseinzedah (2013) showed that sub-bacteriocidal concentrations of oils from *Cinnamomum zeylanicum*, *Thymus vulgaris* (thymol), *Lavandula angustifolia* and *Eucalyptus camaldulensis* suppressed both pathogenicity and virulence factors in race 3 of *R. solanacearum*.

Irrigation water and wastewater from potato processing facilities can spread disease if potato plants are infected. Brown rot was found in Sweden in 1972. The infected field was downstream from two potato factories that released untreated waste water into a river that was used to irrigate potato fields. The potatoes were imported from a Mediterranean origin. A similar situation developed in Belgium in 1989. The Netherlands and other western European countries experienced an outbreak of brown rot in 1995. Contaminated surface water was also involved in this occurrence. (Janse 2012).

Several measures were implemented in Sweden to eradicate *R. solanacearum.* Potatoes were not grown in infected fields for two years. *S. dulcamara*, an alternative host of *R. solanacearum* was removed from waterways. Only certified seed was used and waterways were not used for irrigation. These measures, combined with environmental monitoring for the presence of *R. solanacearum* were successful in eradicating *R. solanacearum* from Sweden (Persson 1998).

van Bueningen et al. (2005) found that 100 ppm of hydrogen peroxide was sufficient to eliminate *R. solanacearum* in irrigation water. It is a common practice for potato processing facilities in the US to add disinfectant, such as chlorine, to their waste water (Sargent, personal communication). However, there is no information on testing for its effectiveness against *R. solanacearum*. Yao et al. (2014) obtained close to 100% inhibition of bacterial growth with 1.3 ppm chlorine dioxide. The strain tested was a pathogen of *Ipomoea aquatica* in Taiwan, so it was probably a race 1 biovar 4 isolate. But it is reasonable to assume that this chemical treatment would also be effective for R3b2. Floyd (2008) describes a protocol for disinfecting irrigation systems using ozonation and UV light. Additional compounds approved for surface disinfection are also discussed by Floyd (2008).

c. Seed priming

Seed priming describes a method used to enhance germination and growth by pre-soaking seeds prior to planting. The potential of seed priming with sodium chloride to induce plant resistance to bacterial wilt was demonstrated by Nakaune et al. (2012). Tomato plants from seeds primed with a solution of 300 mM NaCl showed a greater tolerance to bacterial wilt than plants from hydro-primed seeds or non-primed seeds.

d. Biological control

Research by Aliye et al. (2008) and Lemessa and Zeller (2007) identified bacteria that inhibited bacterial wilt disease in potatoes. 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. Currently, none are commercially available and efficacy of the biological control agents has yet to be determined on a commercial scale. Anaerobic soil disinfestation (ASD), also known as biological soil disinfestation has proven to reduce the level of *R. solanacearum*, including R3b2 in the soil (Messiha et al. 2007; Momma et al. 2013). ASD is a biological pre-plant technique which consists of applying soil amendments, tarping the soil, and then saturating the soil to capacity. Typically the soil is covered for up to three weeks, by which time the environment under the tarp becomes anaerobic (Rosskopf et al. 2015).

e. Bacteriophages

Currently there is only one company, OmniLytics, Inc. (Sandy, UT) that manufactures bacteriophages for biological control of select plant diseases. Although they did manufacture a phage mixture for biocontrol of *R. solanacearum* (Iriarte et al. 2012), it was recently discontinued (OmniLytics, personal communication). Much of the research on use of bacteriophages to control *R. solanacearum* had been for race 1. Therefore, the specific phages may not be directly applicable for use with R3b2. However, the information serves as a useful and relevant model for future work using bacteriophages to control R3b2.

One of the difficulties in using bacteriophages for control of plant diseases is that they do not persist when applied to leaf surfaces due to environmental factors such as sunlight (UV-A and UV-B), high temperatures and desiccation. Sunlight was shown to be the most problematic of all the environmental factors (Jones et al. 2012). Iriarte et al. (2012) demonstrated that when bacteriophages are applied in the rhizosphere, the problem of sunlight is eliminated. Because plants are infected by *R. solanacearum* through the roots, application of bacteriophages into the rhizosphere would be an effective way to deliver bacteriophage treatment to plants.

Tanaka et al. (1990), Tan et al. (2010), Fujiwara et al. (2011), Addy et al. (2012), Iriarte et al. (2012), and Young et al. (2012) all had some success using phages to control *R. solanacearum*, on plants in pots under growth chamber type or greenhouse conditions. Although a great deal of work needs to be done before bacteriophages are commercially available to control R3b2, the potential clearly exists.

f. 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, and quarantine measures on infected fields and farms.

In areas where *R. solanacearum* is already established and widespread, using crop rotation provides the best control. A two or three-year rotation is recommended. Using grasses in the rotation has been successful in reducing the incidence of bacterial wilt (Denny, 2006). One example of a recommended rotation is faba beans-garlic-maize-soybean or wheat. A highly successful rotation used in a study in India included potato-wheat-lupine-maize-potato (Saddler, 2005). Work in East Africa on bacterial wilt in potato demonstrated that different rotation system combinations including pulses, cereals, sweet potatoes cabbage and onions were all effective in decreasing wilt incidence and increasing yield (Lemaga et al. 2005) although the cereals were more effective than the pulses.

Recommended strategies for best management of bacterial wilt of tomato caused by *R. solanacearum* should be followed (Momol et al. 2005). These include 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), or testing and treatment of surface water for irrigation. Integrated application of these strategies to other crops is also critical for successful management of diseases caused by *R. solanacearum*.

g. 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 the bacterium 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, disinfestation of all frames, trays and tools, use of pathogen-free soils or potting mix, control of weeds, and limited handling of plants (McCarter 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.*

XI. Current Infrastructure, Needs and Experts

Studies for initial screening of suspect samples can be carried out by diagnostic laboratories including 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, while important in restricting pathogen spread, also constrain research and the subsequent resulting knowledge and progress in critical areas such as disease control.
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The destructive nature and quarantine status of this pathogen has significantly elevated its importance and the need for effective detection, management, and better understanding of this disease and its causal agent. Therefore, 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 were:

i) to develop rapid, robust, and reliable diagnostic assays for *R. solanacearum* R3b2;
ii) to identify R3b2 genes involved in cold adaptation and growth in plant hosts, using a microarray-based post-genomic approach;
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 July 19, 2016).

R. solanacearum Experts:

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XI. Research, Education and Extension 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:
    a. Develop or screen additional chemical and biological control products.
    b. Exclude the pathogen from potato seeds, geranium cuttings, and tomato transplants, and develop vegetative plant material certification schemes.
    c. Study the effects of cover crops, crop rotation, mulches, and ASD on pathogen dynamics and disease incidence.
2. Develop novel and easy to use detection methods to reliably 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 *R. solanacearum* besides R3b2 have cold tolerance capability. What gene sequence(s) code for cold tolerance? This knowledge might allow regulations directed against all strains of *R. solanacearum* 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 *R. solanacearum* including 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 evaluate their ability to survive in temperate US locations.
6. Develop rapid and inexpensive 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. Develop 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, etc.).
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 cultivars that are 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 agents, 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

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http://www.aphis.usda.gov/plant_health/plant_pest_info/ralstonia/downloads/ralstoniaworkplan.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

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

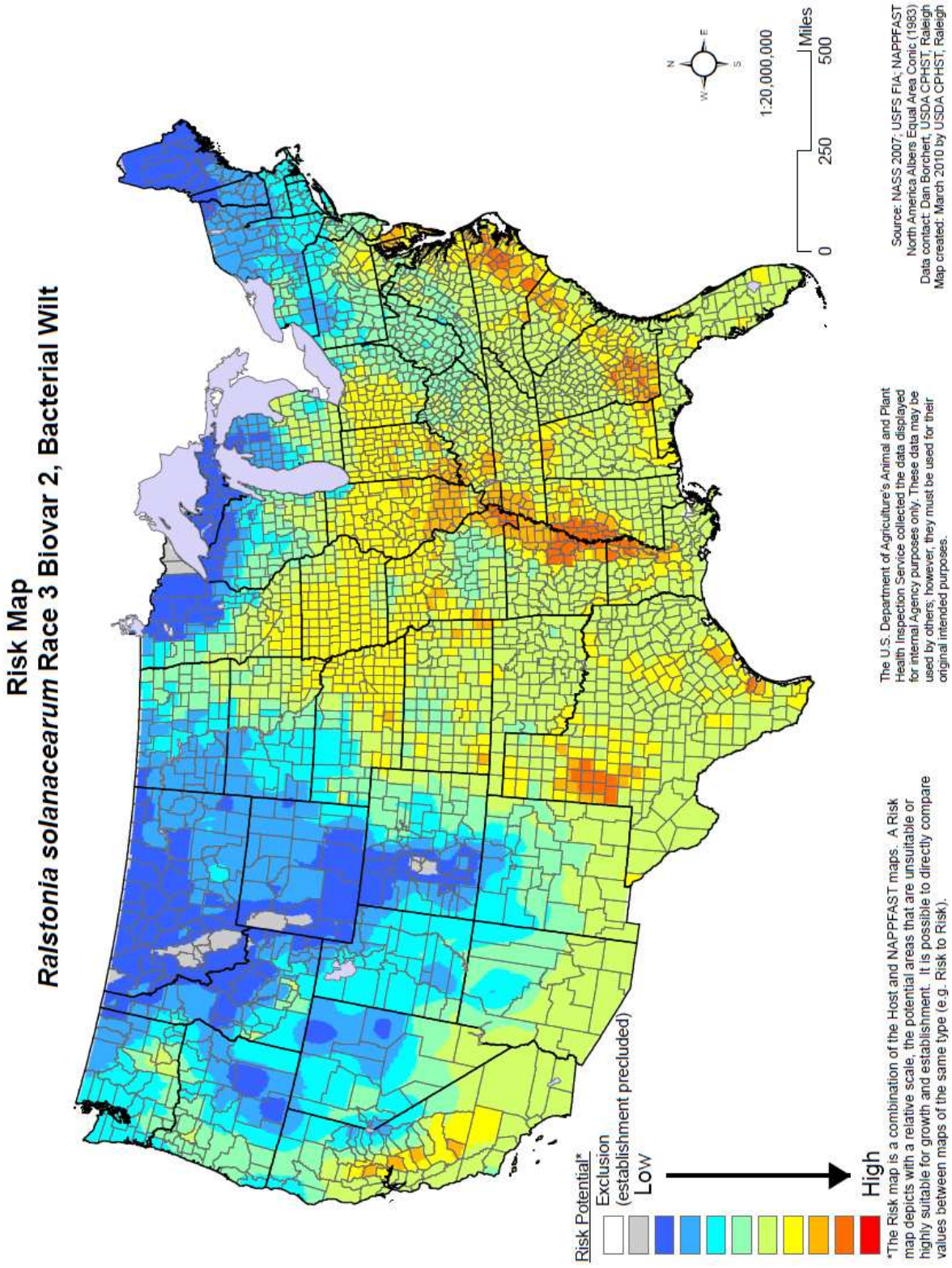
Plant Associated and Environmental Microbes Database (PAMDB) - a multilocus sequence typing and analysis (MLST/MLSA) website and database. http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl

***Ralstonia solanacearum* - National Invasive Species Information Center**
https://www.invasivespeciesinfo.gov/microbes/bacterialwilt.shtml

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

USDA-APHIS Select Agents and Toxins list:
http://www.selectagents.gov/SelectAgentsandToxinsList.html

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