Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

New Pest Response Guidelines

Ralstonia solanacearum “race 3 biovar 2”

Brown rot of potato

Potato wilting due to infection with *R. solanacearum* R3bv2 (Source: Juan Herrera)

R. solanacearum R3bv2

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Introduction

Plant Protection and Quarantine (PPQ) develops New Pest Response Guidelines (NPRGs) in preparation for potential pest introductions. This document is based on the best information available at the time of development and may not reflect the latest state of knowledge at the time the pest is detected. In addition, the PPQ response must be tailored to the specific circumstances of each pest introduction event, which cannot be predicted. Therefore, this document provides only general guidelines that can be used as a basis for developing a situation-specific response plan at the time a new pest is detected.

Program managers of Federal emergency response or domestic pest control programs must ensure that their programs comply with all Federal Acts and Executive Orders pertaining to the environment, as applicable. Refer to the Environmental Compliance section in Appendix A for details.

This document provides information on the select agent *Ralstonia solanacearum* race 3 biovar 2. For more information, refer to the Federal Select Agent Program website.

Chapter

2

Pest Overview

Pest Summary

Ralstonia solanacearum race 3 biovar 2 (R3bv2), a causal agent of potato brown rot, is thought to have originated in the Andean highlands of Colombia and Peru (Champoiseau et al., 2009; Cook and Sequeira, 1991). This pathogen affects mainly solanaceous crops and weeds and is considered a select agent in the U.S. (Janse et al., 2004; USDA-APHIS-PPQ, 2018). A select agent is a biological agent that has the potential to pose a severe threat to plant health (USDA-APHIS, 2017).

Key Information

- Can infect and cause disease at temperatures as low as 61 ºF, but symptoms are most likely at 75–95 ºF).
- Primarily spread through contaminated irrigation and surface runoff water, infested soil, tools and equipment.
- Major hosts are potato, tomato and geranium.
- Many native plant and weed species, such as bittersweet nightshade, can be infected but asymptomatic.
- Main symptoms are wilting, leaf chlorosis (yellowing), stunting, and vascular browning in stems.
- Symptoms of *R. solanacearum* R3bv2 can be mistaken for various other wilt pathogens on potato, tomato and geranium.
- Management in the field is very difficult due to strain variation, host range, pathogen persistence in asymptomatic hosts, infected soil and surface water, and lack of adequate chemical treatments.
- All potentially infected plants and planting material must be destroyed.
- Soil fumigation using chloropicrin has produced similar results to methyl bromide, but further studies are needed.
- Exclusion, use of certified seed, cultural practices and phytosanitation are the best control methods.

Taxonomy

Scientific Name

- *Ralstonia solanacearum* (Smith, 1896) Yabuuchi et al., 1996 race 3 biovar 2
- A revised classification system based on phylogenetic analysis of genome sequences places *Ralstonia solanacearum* R3bv2 strains in sequevars 1 and 2 of the phylotype II B subgroup (Allen et al., 2005; Prior and Fegan, 2005b).

Taxonomic Position

- Bacteria : Proteobacteria : Betaproteobacteria : Burkholderiales : Burkholderiaceae

Synonyms

- *Burkholderia solanacearum* (Smith, 1896) Yabuuchi et al.
- *Pseudomonas solanacearum* (Smith, 1896) Smith

Common Names

- Brown rot of potato
- Bacterial wilt of tomato
- Southern wilt of geranium

Biology and Ecology

Life Cycle

Ralstonia solanacearum R3bv2 is soil and waterborne and can survive for days to years in infected soil (at least 29 inches) (Champoiseau et al., 2009; Graham et al., 1979), as well as surface irrigation water and weeds (Champoiseau and Momol, 2009). This bacterium thrives in cool tropical highlands and in temperate zones. *Ralstonia solanacearum* R3bv2 is highly virulent at temperatures between 66 ºF and 82 ºF (Huerta et al., 2015). Virulence decreases with temperatures above 95 ºF or below 60.8 ºF (Champoiseau et al., 2009; Ciampi and Sequeira, 1980). Additionally, Milling et al. (2009) found that the bacterium can survive for more than 4 months in sterile water at 39 ºF. Other factors affecting disease development include soil type and structure, soil moisture, salt content and water pH (Champoiseau and Momol, 2009).

Infection can occur through wounds in roots (points of emergence of lateral root), injury caused by soil-borne organisms (e.g. the root-knot nematode) and stem injuries caused by agricultural practices (Champoiseau and Momol, 2009; Swanson et al., 2005). Once the plant is infected, the pathogen spreads systemically through the xylem vessels causing wilting and death (Champoiseau et al., 2009; Genin, 2010). Symptomless infection is common, especially at cooler temperatures.

Hosts

Ralstonia solanacearum R3bv2 has a broad host range (Table 2-1) that includes many asymptomatically infected native plant and weed species (Jones et al., 2017). In Europe, numerous outbreaks of *R. solanacearum* R3bv2 have been attributed to bittersweet nightshade, a weed species that grows along waterways and in wet areas (Janse, 1996). Bittersweet nightshade is present in the U.S. and considered an invasive species (Waggy, 2009). Tables 2-2 and 2-3 list other species reported to be asymptomatic hosts.

Table 2-1 List of reported plant hosts of *R. solanacearum* R3bv2

Scientific name	Common name	References
Amaranthus viridis	green amaranth	Lin et al. (2015)
Capsicum annuum	sweet pepper	Martin and French (1995)
Capsicum spp.	Pepper	Lin et al. (2015)
Oxalis latifolia	broadleaf wood-sorrel	Khoodoo et al. (2010)
Pelargonium × *hortorum*	zonal geranium	Williamson et al. (2002)
Pelargonium spp.	Geranium	Ozakman and Schaad (2003)
Pelargonium zonale	horseshoe pelargonium	Janse et al. (2004)
Physalis angulate	cut-leaf ground-cherry	Swanepoel (1992)
Portulaca oleracea	common purslane	Lin et al. (2015)
Solanum americanum	American nightshade	Khoodoo et al. (2010)
Solanum betaceum (=*Cyphomandra betacea*)	tree-tomato	Martin and Nydegger (1982)
Solanum cinereum	Narrawa-bur	Graham and Lloyd (1978)
Solanum lycopersicum	Tomato	Mahbou Somo Toukam et al. (2009)
Solanum melongena	Eggplant	Caffier and Hervé (1996)
Solanum nigrum	black nightshade	Tomlinson and Guntber (1986)
Solanum pimpinellifolium (=*Lycopersicon pimpinellifolium*)	currant tomato	Khoodoo et al. (2010)
Solanum spp.	Potato	Allen et al. (2005)
Solanum tuberosum	Potato	Tahat and Sijam (2010)
Solanum tuberosum subsp. *andigenum* (=*Solanum phureja*)	Andean potato	Ciampi and Sequeira (1980); Patil et al. (2012)

Table 2-2 List of reported asymptomatic hosts of *R. solanacearum* R3bv2

Scientific name	Common name	References
Ageratum conyzoides	Goatweed	Tusiime et al. (1998)
Amaranthus spp.	Pigweed	Tusiime et al. (1998)
Bidens pilosa	beggar-ticks	Tusiime et al. (1998)
Dopatrium sp.	Dopatrium	Pradhanang and Momol (2001)
Drymaria cordata	tropical chickweed	Pradhanang et al. (2000)
Erigeron floribundus	Bilbao fleabane	Tusiime et al. (1998)
Galinsoga parviflora	dumb-nettle	Pradhanang and Elphinstone (1996); Tusiime et al. (1998)
Leucas martinicensis	Whitewort	Tusiime et al. (1998)
Monochoria vaginalis	pickerel-weed	Pradhanang and Momol (2001)
Nicotiana glutinosa	NA	Fernandez (1986)
Oxalis latifolia	broadleaf wood-sorrel	Tusiime et al. (1998)
Persicaria capitata (=*Polygonum capitatum*)	Japanese knotweed	Pradhanang and Elphinstone (1996); Pradhanang et al. (2000)
Persicaria nepalensis (=*Polygonum nepalense*)	Nepal knotweed	Tusiime et al. (1998)
Rumex abyssinicus	Mekmeko	Tusiime et al. (1998)
Solanum dulcamara	bittersweet nightshade	Wenneker et al. (1999)
Stellaria sennii	NA	Tusiime et al. (1998)
Spergula arvensis	corn spurrey	Tusiime et al. (1998)
Tagetes minuta	Aztec marigold	Tusiime et al. (1998)
Urtica dioica	stinging nettle	Wenneker et al. (1999)

Table 2-3 List of hosts of *R. solanacearum* R3bv2 that can be asymptomatically infected under artificial conditions

Scientific name	Common name	References
Brassica juncea	brown mustard	Pradhanang et al. (2000)
Brassica oleracea	Cabbage	Álvarez et al. (2008)
Brassica spp.	wild mustard	Álvarez et al. (2008)
Calibrachoa sp.	Calibrachoa	Janse et al. (2004)
Cerastium glomeratum	mouse-ear chickweed	Pradhanang et al. (2000)
Cichorium endivia	Endive	Álvarez et al. (2008)
Datura stramonium	Jimsonweed	Fernandez (1986)
Nicandra physalodes	broadleaf-nightshade	Pradhanang et al. (2000)
Nicotiana glutinosa	knekt-tobak	Martin and French (1995)
Nicotiana rustica	Aztec tobacco	Martin and French (1995)
Petunia × *atkinsiana* (=*Petunia* × *hybrida*)	common garden petunia	Janse et al. (2004)
Physalis pubescens (=*Physalis floridana*)	downy ground-cherry	Fernandez (1986)
Salpiglossis sinuata	painted-tongue	Olsson (1976)
Solanum pseudocapsicum (=*Solanum capsicastrum*)	false capsicum	Fernandez (1986)
Solanum virginianum (=*Solanum xanthocarpum*)	yellow-fruit nightshade	Pradhanang et al. (2000)
Stellaria media	Chickweed	Pradhanang et al. (2000)
Tropaeolum majus	garden nasturtium	Pradhanang et al. (2000)

Dispersal

Natural Movement

Ralstonia solanacearum R3bv2 can spread naturally from infected roots to healthy roots of neighboring plants (Kelman and Sequeira, 1965 as cited by Kelman, 1998) and through insect and possibly nematode damage (Champoiseau et al., 2009). Note that this pathogen does not spread from plant to plant aerially or through casual contact or water splashing (Swanson et al., 2005).

Human-Assisted Spread

This pathogen is primarily water and soilborne and can be dispersed by contaminated irrigation and surface runoff water, infected soil and plant material, soil transfer on machinery and equipment, and unsanitized handling (Champoiseau and Momol, 2009; Janse, 1996). Greenhouse spread may occur through transplanting infected plants, pinching buds off plants without sanitizing (Jones et al., 2017), using contaminated tools between cuttings (Janse et al., 2004) and irrigating with sub-irrigation or ebb-and-flow systems (Swanson et al., 2005). Vegetative propagation also plays a key role in spreading *R. solanacearum* R3bv2 through asymptomatically infected seed tubers and cuttings of geranium and other ornamentals (Janse, 1996; Jones et al., 2017).

According to studies by Pasqua di Bisceglie et al. (2005), *R. solanacearum* can survive on poplar for 17 days, oak for 4 days, and on high-density polyethylene in cold storage at 39 °F and 80–90% relative humidity for 2 days. Survival of *R. solanacearum* on jute fabric dropped after 24 hours with the population reaching zero after 78 days.

Geographic Distribution

World Distribution

Table 2-4 Reported worldwide distribution of *R. solanacearum* R3bv2

Country	References
Argentina	French (1988)
Australia	Graham and Lloyd (1979); Stansbury et al. (2001)
Bangladesh	Chakraborty and Roy (2016)
Belgium[1]	EPPO (2015b)
Bolivia	Castillo and Plata (2016)
Brazil	Almeida et al. (2003)
Burundi	Autrique and Potts (1987)
Cameroon	Mahbou Somo Toukam et al. (2009)
Chile	Ciampi et al. (1997); van der Wolf et al. (2004)
China	Wang et al. (2017)
Colombia	Lebeau et al. (2011); Prior and Fegan (2005a)

Country	References
Costa Rica	Gabriel et al. (2006); Williamson et al. (2002)
Czech Republic[1]	EPPO (2012a)
Egypt	Farag et al. (1999)
Ethiopia	Lemessa and Zeller (2007)
France[1]	EPPO (2014)
French Guiana	Deberdt et al. (2014)
Georgia	Lashkhi et al. (2018)
Germany[1]	EPPO (2012b)
Greece[2]	EPPO (2007)
Guadeloupe	Prior and Steva (1990)
Guatemala	Sanchez Perez et al. (2008)
Hungary[2]	EPPO (2002); Nemeth et al. (2002)
India	Sagar et al. (2013)
Indonesia	Horita and Tsuchiya (2001)
Iran	Izadiyan and Taghavi (2013)
Japan	Horita and Tsuchiya (2001)
Kenya	Janse et al. (2004)
Korea, Republic of	Jeong et al. (2007)
Malawi	Zayamba Kagona (2008)
Mauritius	Khoodoo et al. (2007)
Mexico	Hernández-Romano et al. (2012); Perea Soto et al. (2011)
Nepal	Pradhanang et al. (2000)
Netherlands	EPPO (2016)
New Caledonia	EPPO (2015c); IPPC (2015)
Nigeria	Popoola et al. (2015)
Pakistan	Begum (2011)
Papua New Guinea	Tomlinson and Guntber (1986)
Peru	Gutarra et al. (2017)
Philippines	Natural et al. (2005)
Poland[1]	EPPO (2015a)
Portugal	Cruz et al. (2012)
Reunion	Nicole et al. (1998)
Russia	Matveeva et al. (2003)
Rwanda	Uwamahoro et al. (2018)
Slovakia[1]	EPPO (2004)
South Africa	Cellier et al. (2012)
Spain	Caruso et al. (2017)
Sri Lanka	EPPO (1998)
Sweden[1]	EPPO (2010)
Taiwan	Wu et al. (2011)
Tanzania	Mwankemwa (2015)
Turkey	Ustun et al. (2008)
Uganda	Allen et al., 2005
Uruguay	Siri et al. (2011)
Venezuela	Garcia et al. (1999)

[1] Under eradication

[2] Under official control

Potential Distribution in the United States

Ralstonia solanacearum R3bv2 is not known to be present in the U.S. However, nearly every state grows at least one of the major crops that can host *R. solanacearum* R3bv2. California, Colorado, Idaho, Maine, Michigan, Minnesota, North Dakota, Oregon, Washington and Wisconsin harvest the majority of potatoes (Fig. 2-1) (USDA–APHIS–PPQ–S&T Fort Collins Lab, 2018a), while California and Florida are the major producers of tomatoes (Fig. 2-2) (USDA–APHIS–PPQ–S&T Fort Collins Lab, 2018b). Geraniums, sold as annual bedding/garden plants, are grown in California, Florida, Hawaii, Illinois, Maryland, Michigan, New Jersey, New York, North Carolina, Ohio, Oregon, Pennsylvania, South Carolina, Texas and Washington (USDA-NASS, 2016). Figure 2-3 includes combination map of potato and tomato.

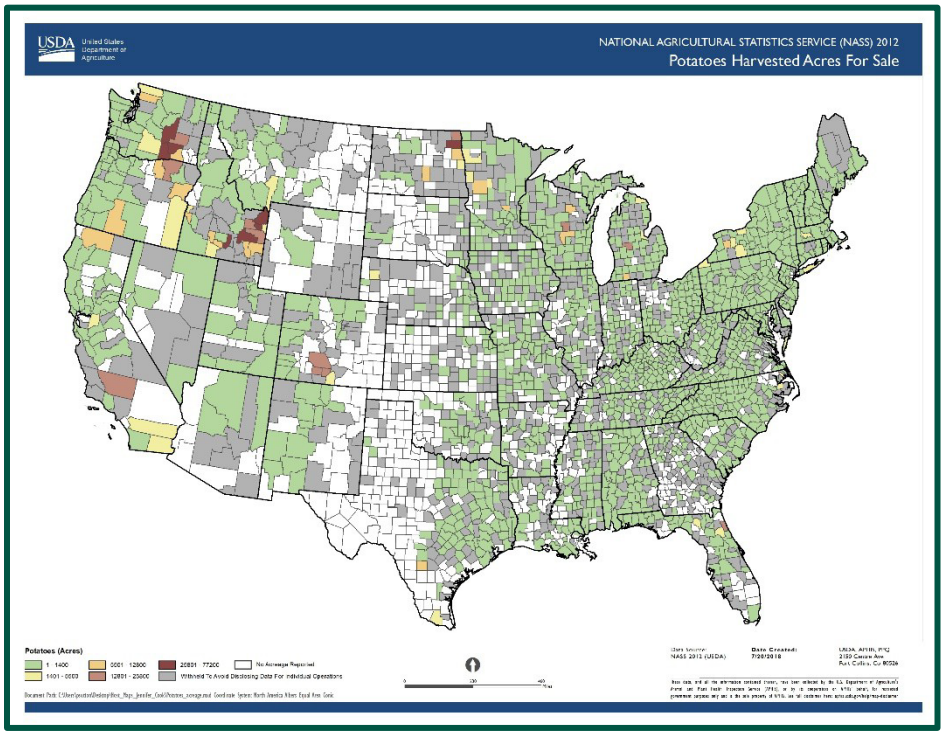

Figure 2-1 Potatoes harvested acres for sale in the continental U.S. in 2012 (Source: USDA–APHIS–PPQ–S&T Fort Collins Lab, 2018a)

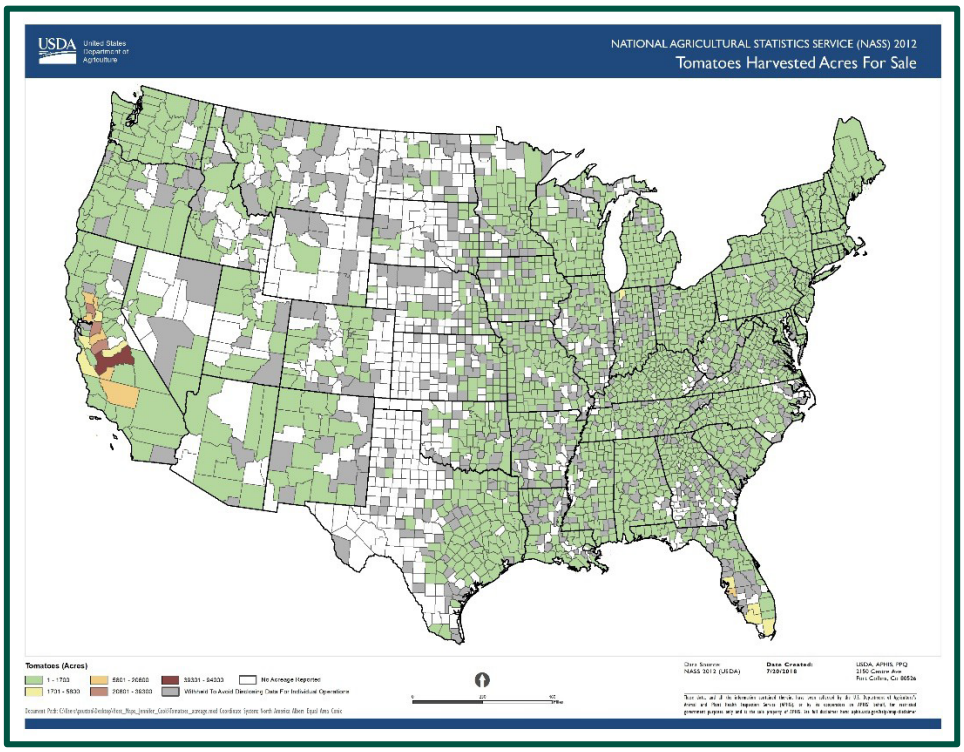

Figure 2-2 Tomatoes harvested acres for sale in the continental U.S. in 2012 (Source: USDA–APHIS–PPQ–S&T Fort Collins Lab, 2018b)

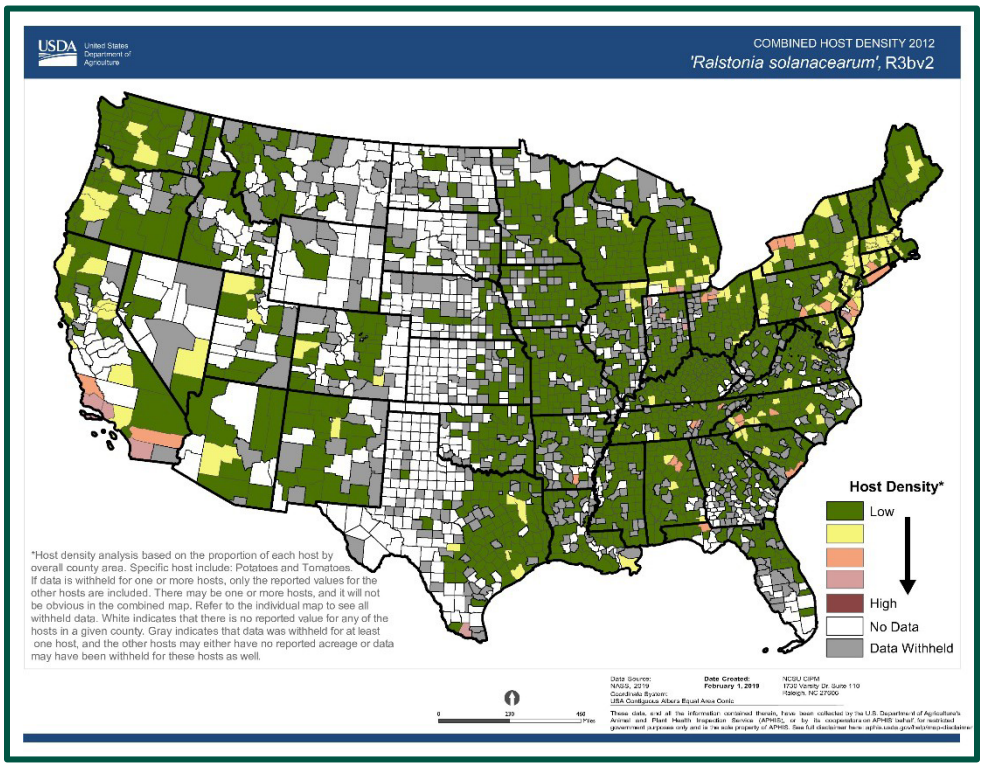

Figure 2-3 Combined host density of potato and tomato in the continental U.S. in 2012 (Source: NCSU-CIPM, 2019)

Chapter 3

Pest Identification and Damage

Species Description/Morphology

Ralstonia solanacearum R3bv2 is a gram-negative, rod-shaped, motile, strictly aerobic bacterium that is 0.2–0.28 inches × 0.59–0.79 inches in size (Champoiseau et al., 2009; Smith, 1896; Yabuuchi et al., 1995).

Normal or virulent type colonies are irregularly shaped, white or cream-colored, opaque and highly fluidal, while the mutant or non-virulent type colonies appear consistently round, smaller, butyrous (buttery consistency) or dry (Champoiseau et al., 2009). *Ralstonia solanacearum* R3bv2 grows relatively slowly and is easily outcompeted by other microbes in culture; individual bacterial colonies that appear in less than 36 hours are not *R. solanacearum* (Allen, 2019).

ID/Diagnostic

Molecular

- Real-time, multiplex PCR assay, specifically an assay for biovar 2A, can detect all strains of *R. solanacearum* (USDA-APHIS-PPQ-CPHST, 2015; Weller et al., 2000).
- An additional multiplex reaction was developed for infected potato tissue that integrates a third primer set with an internal control (USDA-APHIS-PPQ-CPHST, 2015).
- If a sample tests positive for *R. solanacearum*, Select Agent Program guidelines must be followed. See Appendix B for further information.

Signs and Symptoms

Ralstonia solanacearum R3bv2 symptoms are identical to those from other *R. solanacearum* strains (Champoiseau, 2009). Additionally, under favorable conditions both geranium and potato plants may form asymptomatic infections (Swanson et al., 2005). Signs and symptoms of *R. solanacearum* R3bv2 include the following:

Potato and Tomato

- Early symptoms of wilting occur on the youngest leaves during the hottest time of the day. Wilting may be limited to the top portion of the plant on just one side of a leaflet or an individual branch. Plants may appear to recover following rain or when temperatures cool down at night (Fig. 3-1) (Champoiseau et al., 2009). Infected tomato plants often develop adventitious roots on the lower stem (Allen, 2019).
- The entire plant may decline rapidly under favorable conditions, starting with wilt, leaf chlorosis and ending with death. Leaves can wither, but dried leaves remain green (Champoiseau et al., 2009). In tomato, disease develops rapidly and plants may die within 4–7 days after the first appearance of wilt symptoms (Jones et al., 2017).
- Stunting is another common symptom, occurring at any stage of growth (Champoiseau et al., 2009).
- Infected stems may collapse, revealing vascular browning displayed as narrow, dark brown necrotic streaks with grey-white bacterial ooze (Fig. 3-2) (Champoiseau et al., 2009). When disease develops very rapidly, ooze may appear on the surface of intact stems (Allen, 2019).
- In symptomatic potato tubers the vascular ring turns a grey-brown color that may extend into the pith or cortex as the infection progresses. When infected potatoes are cut open, they ooze a milky-white sticky exudate. Visible threads may form from the ooze when the two sides of a cut potato are pressed together and then pulled apart. This ooze may also cause dirt to adhere to the tuber (Fig. 3-3) (Champoiseau et al., 2009).
- A common diagnostic sign is bacterial streaming, which occurs when freshly cut stems from infected plants are placed in water. Fine, milky white strands of a viscous white slime containing bacteria often run from the cut end of the stem within 15 minutes (Fig. 3-4) (Jones et al., 2017).
- Bacterial streaming and oozing may not be visible in the early stages of disease development (Jones et al., 2017).

Geranium

- Early wilt symptoms in geranium are subtle and sometimes unnoticeable. Symptoms begin with chlorosis and wilt in the lower leaves and progress into upward curling of leaf margins (Figs. 3-5 and 3-6) (Champoiseau et al., 2009).
- Like potato and tomato, geraniums may appear to recover in the cooler night temperatures (Champoiseau et al., 2009).
- The disease develops rapidly as wilting moves upward from older to younger leaves (Champoiseau et al., 2009).

- Wilted leaves often have wedge-shaped areas of chlorosis that become necrotic (Champoiseau et al., 2009).
- In later stages of disease, stem collapse can occur. Stems (particularly at the root crown) and roots exhibit vascular discoloration, which can blacken and eventually become necrotic (Champoiseau et al., 2009).
- Bacterial streaming can also occur in geranium (Champoiseau and Momol, 2009).

Figure 3-1 Wilting on potato plant caused by infection with *R. solanacearum* R3bv2 (Source: Amilcar Sanchez Perez)

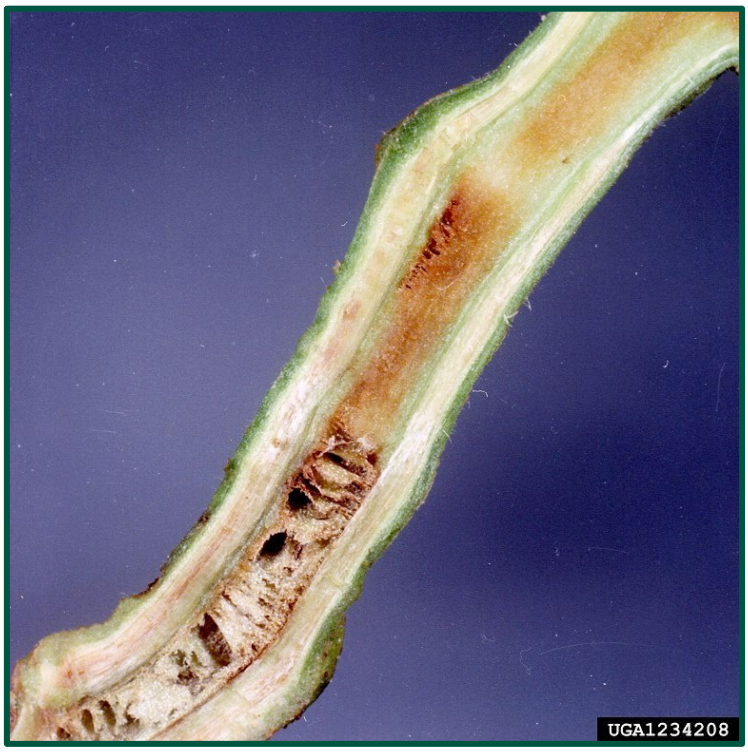

Figure 3-2 Cut section of a tomato stem displaying vascular browning caused by infection with *R. solanacearum* (Source: Clemson University - USDA Cooperative Extension Slide Series, Bugwood.org)

Figure 3-3 Cut section of potato tubers displaying a brown discoloration to the vascular ring caused by infection with *R. solanacearum* R3bv2 (Source: Caitilyn Allen, University of Wisconsin-Madison)

Figure 3-4 Bacterial ooze on geranium caused by infection with *R. solanacearum* R3bv2 (Source: Margery Daughtrey, Cornell University, Bugwood.org)

Figure 3-5 Left: Chlorosis on geranium plants caused by infection with *R. solanacearum* R3bv2; Right: Healthy geranium (Source: Caitilyn Allen, University of Wisconsin-Madison)

Figure 3-6 Early wilting symptoms on geranium caused by infection with *R. solanacearum* R3bv2 (Source: Jean L. Williams-Woodward, University of Georgia, Bugwood.org)

Similar Species

Other diseases that produce symptoms that can be mistaken for *R. solanacearum* R3bv2 include the following:

- Other *R. solanacearum* strains that are endemic in the southern U.S. produce indistinguishable symptoms in the same hosts (Jones et al., 2017).
- *Xanthomonas hortorum* pv. *pelargonii*, the causal agent of bacterial blight of geranium, produces wilting symptoms similar to *R. solanacearum* R3bv2 (Champosiseau et al., 2010).
- *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of *Fusarium* wilt on tomato, causes wilting and chlorosis on one side of the plant and dark brown streaks in the stem (Cook and Sequeira, 1991; Jones et al., 1991).
- *Verticillium albo-atrum* and *V. dahliae*, the causal agent of *Verticillium* wilt on potato and tomato (Cook and Sequeira, 1991; Jones et al., 2017), causes leaf wilting during the day, which reverses at night (tomato), chlorosis on one side of the plant (potato) and vascular browning in infected stems (potato and tomato) (Jones et al., 1991; Stevenson et al., 2001).
- *Clavibacter michiganensis* subsp. *sepedonicus*, causal agent of potato ring

rot, produces a vascular ring containing bacterial ooze and causes leaf wilting during the hottest period of the day and looks like the plant recovers overnight (Stevenson et al., 2001).

Abiotic stresses, such as drought, mechanical damage to the root or nutrient deficiency can also produce symptoms easily mistaken for *R. solanacearum* R3bv2. Therefore diagnosis should not be based solely on symptoms (Jones et al., 2017).

Chapter

4

Delimitation Survey

Delimitation Area

Delimitation surveys determine the extent of the infected area after an infection has been confirmed. Since each survey location has different characteristics and agency resources are unknown until a response begins, PPQ develops situation-specific surveys after pests are detected. To prepare response teams for the delimitation survey, we provide survey considerations below.

Survey Techniques for Delimitation

Survey Preparation, Sanitization and Clean-Up

1. Prior to beginning a survey, determine whether there have been recent pesticide applications that would render it unsafe to inspect the plants and soil. Contact the property owner or manager and ask if there is a re-entry period in effect due to pesticide application. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or nurseries.
2. Determine whether quarantines for other pests or crops are in effect for the survey area. Comply with all quarantine requirements.
3. When visiting the area to conduct surveys or take samples, take strict measures to prevent contamination by *R. solanacearum* R3bv2 or other pests between properties during inspections.
4. Designate a clean area where transport vehicles can park. Make sure this area is not located near infected fields.
5. **Important**: Use disposable protective clothing, gloves and footwear, and change them before entering each site. Disinfectants available for use on skin are listed in Table 4-1.
6. Disinfect all potentially contaminated surfaces (i.e. benches, flats, walkways, footbaths, drainage areas under benches, footwear) and equipment near an infected greenhouse or area that may have come in contact with infected material (Table 4-2).
    a. Clean any soil or media adhering to the surface.
    b. Change the disinfectant in footbath reservoirs at least twice daily

(USDA-APHIS, 2007).

c. When taking plant samples, disinfect tools with an approved surface disinfectant prior to and between uses (Table 4-2). Submerge the entire blade or portion of that tool that makes is in contact with soil or plant material.

d. **Important**: To disinfect large pieces of equipment, storage areas or bins, use a high-pressure delivery system, such as a steam pressure wash system, at 212 ºF.

7. Drain and clean all recirculating irrigation systems, sub irrigation systems and any systems that do not prevent backflow of water from infected greenhouses.

a. Clean all parts, sumps, and pumps, with approved disinfectant solutions. To be effective against *R. solanacearum* R3bv2, systems must have ozonation with 0.4 ppm residual ozone for four minutes with ultraviolet (UV) light of at least 300 j/m^2 (1.36 ft-lb/in^2) at >50% transmission (USDA-APHIS, 2007).

8. When ponds, outdoor soil or holding areas have become contaminated during plant storage or runoff, contact your PPQ regional office and APHIS State Plant Health Director (SPHD) about possible environmental consideration and treatment options.

Table 4-1 Approved skin disinfectant active ingredients for *R. solanacearum*. Follow product label instructions prior to using any product (USDA-APHIS, 2007)

Active Ingredient
Alcohols (>60% ethanol)
Chlorhexidine (0.5%-4.0%)
Chloroxylenol (0.3%-3.75%)
Iodine and iodophors (7.5%-10.0% povidone-iodine)
Quaternary ammonium
Triclosan (0.2%-2.0%)

Table 4-2 Approved surface disinfectant active ingredients for *R. solanacearum*. Follow product label instructions prior to using any product (USDA-APHIS, 2007)

Active Ingredient	Use Sites
Chlorine	Greenhouse hard non-porous surfaces (i.e. floor, walls, benches, counter tops, buckets, coolers) Shoe wash
Iodine	Greenhouse hard non-porous surfaces (i.e. floor, walls, benches, counter tops, buckets, coolers) Shoe wash
Quaternary ammonium (ammonium chloride)	Outer clothing, field harvesting equipment, greenhouse packing areas Greenhouse hard non-porous surfaces (i.e. floors, walls, benches, counter tops, buckets, coolers, tools, metal and plastic surfaces, knobs, handles, railings, glass) Walkways

Visual Inspection

Conduct a visual inspection by searching for plants with typical wilting symptoms (see Signs and Symptoms). The absence of symptoms, however, does not confirm an absence of *R. solanacearum* R3bv2 in the inspected area. Some infected plants may be asymptomatic, even when continually exposed to ideal temperatures. Other areas to survey include the following:

- Look for signs of wilting in areas where water accumulates or throughout the field. *Ralstonia solanacearum* R3bv2 is readily carried in irrigation water and can spread rapidly.
- Inspect plants, including weeds, near drainage canals or irrigation rigs.

Delimitation Survey

Survey timing should depend upon the pathogen life cycle, the plant growth stage when infection is likely to occur, and ecological parameters that support pathogen dispersal. Other considerations include logistics and available resources; available resources can vary based on the time and location of detection and the pest species.

Prior to collecting samples:

- Define the site to be sampled on an acre basis.
- For whole plant sampling use the following or current surveying procedures already set in place in your state or county:
    - Walk down rows and collect symptomatic plants.
    - For example: Use the following to determine sampling rows (NDSU, 2018):
        - If plants are widely spaced, 36 inches apart, walk down every 6th row looking at all plants in adjacent rows up to 20 feet out.
        - If they are tightly spaced, 12 inches apart, do the same every 20th row.
    - Take random samples to represent the entire acre.

Sample Collection

Surveyors visiting sites to place holds or obtain samples should collect the

following information:

- Date of collection or observations.
- Collector's name.
- Grower's field identification numbers (grower can provide this information).
- Full name of business, institution, or agency.
- Full mailing address, including county.
- Type of property (commercial nursery, natural field, residence).
- GPS coordinates of the survey site.
- Host plant species and specific crop plant variety, if applicable.
- Presence or absence of the pest.
- Observations of signs and symptoms.
- Percentage of the field displaying disease symptoms.
- General conditions or any other relevant information.

Test symptomatic plants for the presence of *R. solanacearum* bacteria using ImmunoStrip® available from commercial providers. Positive reactions will indicate the presence of *R. solanacearum*, but will not distinguish the R3bv2 subgroup (USDA-APHIS-PPQ-CPHST, 2018). If a sample is positive, follow the USDA Select Agent Process listed in Appendix B:

- Collect entire plants (including roots) showing wilting symptoms and wash the soil from them.
- At a minimum, enclose 0.04 ounce of symptomatic stem/crown tissue.
- Double bag the samples and keep cool. **Do not refrigerate.** Samples may be held at room temperature if less than or equal to 60 ºF.

Tuber Sampling

Sampling is **not** required for fields or facilities with a direct link to positive testing seed lots or fields. Tubers in this category are automatically considered positive and are subject to control (quarantine) actions without testing.

1. Sampling for subsequent testing is required for tubers:
    a. In adjacent fields.
    b. On a shared water source.
    c. Connected by a history of shared harvesting machinery.
2. Follow these guidelines for sample collection:
    a. Samples include potato tubers, stems from potato or other host plants, and seed pieces (Elphinstone et al., 2018).
    b. Repeat the sampling for each field, warehouse or storage unit at a given location.

c. Collect a maximum of 200 samples from each location (Elphinstone et al., 2018).
d. Collect samples throughout the entire building or storage area.
e. If tubers are in bags, collect samples from each bag.
f. Double bag the samples and keep cool. **Do not refrigerate.** Samples may be held at room temperature if less than or equal to 60 ºF.
g. Prepare samples within 72 hours of collecting the sample.

Tomato and Other Solanaceous Hosts

1. Collect entire plants showing wilting symptoms, including roots, and wash them free of soil. Bare root plants are ideal. Since the pathogen is concentrated in the lower stem, leaf and partial stem samples are inadequate for testing.
   a. Double bag the samples and keep cool. **Do not refrigerate.** Samples may be held at room temperature if less than or equal to 60 ºF.
   b. Submit entire plants, not sub-samples. Samples must include lower stems with the leaves and petioles removed. Samples that are dead or fermented upon arrival cannot be tested and will be rejected.

Sampling of Water Sources

1. Test irrigation and water sources near positive fields or processing plants to limit the bacterium's spread. At present, there is no test that can be used in the field. Therefore, all water samples must be sent to a diagnostics lab.
2. When taking samples:
   a. Sterilize bottles.
   b. Collect samples of approximately 0.50 quarts.
   c. Collect water samples at a depth of 12 inches.
   d. Keep samples cool and in a dark location and perform tests within 24 hours of collection.
   e. For best results, conduct sampling when water temperatures exceed 59 ºF and populations of bacterium are highest in water(Jones et al., 2017).

Sample Shipping

See Appendix B for USDA-APHIS procedures for submitting domestically detected suspect *R. solanacearum* positives based on immunostrip testing.

Timing of Surveys

In general, survey for this pathogen should take place during the day when temperatures are the hottest and symptoms of wilt, if present, are most obvious.

Chapter

5

Eradication and Control Options

Overview

This information can be used by PPQ decision-makers after a detection to assess the suitability of potential actions to eradicate, contain, or suppress *R. solanacearum* R3bv2. Although eradication should always be prioritized, its success will depend on invasion factors unique to this pathogen.

Eradication Options

Quarantine and Regulatory Procedures

Remove all suspected or potentially infected plants (i.e., potato, tomato, geranium, and weeds (to include aquatic)) (see Host Removal).

Dispose of all host plant material on any property that tests positive or has positive-associated fields, including cull piles and other plant debris.

Leave the field fallow for two years and irrigate to promote volunteer sprouting. During the two years, sample, test, cull and bury under the volunteer crop during the growing season every four weeks to eliminate host material. Spray weed hosts in the field and along the edges with efficacious labeled herbicides to eliminate them from the area.

Following the two-year period, plant fields with non-host crops; irrigate to promote volunteer potato sprouting, and cull and bury any volunteer potatoes as sprouting occurs. Test fields semi-annually for four years after an initial positive find.

No seed production can occur in the field for at least five years after detection of *R. solanacearum* R3bv2. Sample fields with susceptible hosts (potatoes or tomatoes) for two seasons prior to any new seed production on the property.

Fields adjacent to positive testing or associated fields, or those on a shared water

supply may **not** grow host crops **nor** seed potatoes for two years. Any volunteer potato or weed hosts must be tested and destroyed. Maintain strict sanitation of all vehicles entering and leaving infested fields.

Quarantine storage facilities on properties with positive testing tubers until all potentially infested tubers are tested and found to be negative or destroyed. Clean and disinfect storage facilities with approved disinfectants in strict accordance with labeling.

Host Removal

Place all potentially infected plants and planting material, as determined by the delimiting survey, in double plastic bags and seal for disposal or destruction. If large inventories must be destroyed, then use of plastic bags may not be reasonable. Alternatives include a dumpster with double layers of plastic lining that can be folded over the top and sealed to prevent debris from escaping during transport or storage. Contact your regional PPQ office.

Incineration and steam sterilization are the preferred methods of disposal; however, these options may **not** be practical for large amounts of waste. In these cases, use an approved landfill.

Incinerate all plant material and media to the point of ash. If plastic pots are **not** accepted at the incinerator, remove them and properly disinfect or send to an approved landfill (see below).

Soil fumigation with an approved fumigant—such as methyl bromide or metam sodium—at the labeled rate will greatly reduce populations of the bacteria in infested fields. However, fields with plant residue including tubers, roots and stems may still harbor *R. solanacearum* R3bv2 for up to two years. Before fumigation, remove and destroy as much host plant material from the fields as possible.

Fumigation of the soil may **not** totally eliminate the pathogen due to the presence of bacterial reservoirs in buried plant residue at the time of fumigation. Continue monitoring the soil for the presence of *R. solanacearum* R3bv2. Select agent regulations need to be followed if *R. solanacearum* is detected.

If an approved landfill is used, bury the double bagged material from nurseries or potato cropping systems under at least 6.6 feet or more of soil (Ebbels, 2003).

Alternative Control Techniques

Chemical Control

Managing *R. solanacearum* in the field is very difficult due to strain variation, broad host range and lack of adequate chemical treatments (Jones et al., 2017). Listed below are three chemical control options shown to be effective against *R. solanacearum* R3bv2 experimentally, but they have not been tested on a larger scale.

DL-3-aminobutyric acid (BABA) is an abiotic compound reported to induce resistance in tomato plants. When applied by soil drenching to tomato plants at 0.05 quarts per pot, it reduced the vascular browning index by 69.9% and leaf wilting index by 75.3% (Hassan and Abo-Elyousr, 2013).

Stable bleaching powder (SBP) applied two weeks before planting at a rate of 22.3 pounds per acre suppressed *R. solanacearum* R3bv2 by 76.94% and 88.89% for tuber infection and 66.96% and 71.87% for plant infection in greenhouse and field studies, respectively (Dhital et al., 1997).

During field trials on ginger, Mao et al. (2014) tested for alternatives to methyl bromide against infection with a phylotype I (Asian) *R. solanacearum* strain. Chloropicrin was injected at 1.47 ounces per yard^2 and covered with either a 0.002-inch polyethylene film (PE) or a 0.002-inch totally impermeable film (TIF), while methyl bromide was applied at a rate of 1.18 ounce per yard^2 in situ under a PE sheet. Both treatments of chloropicrin produced similar efficacies to, or in some cases slightly lower than, methyl bromide in terms of controlling *R. solanacearum* R4bv4 infection.

Labeling

Although a proposed formulation may be approved by APHIS as an effective eradication or chemical control program, it may not be labeled at the time of pest detection for the specific use site or rate required. If a formulation is not labeled for the necessary use, there may be several options. One can request a quarantine exemption from the EPA under section 18 of FIFRA, or a Section 24c from the state where the product will be applied. The prescribed formulation must be labeled both for use on the site at which it is to be applied and at the desired rate and must be registered for use in the state in which the eradication program is occurring. All applicable label directions must be followed, including but not limited to requirements for personal protection equipment, maximum treatment rates, storage and disposal.

Cultural Control and Sanitary Measures

Cultural practices and phytosanitation are the best control measures for *R. solanacearum* R3bv2 in the field(Jones et al., 2017).

Crop rotation every two or three years provides the best control in areas where *R. solanacearum* is already established and widespread (Jones et al., 2017). A two-season rotation with bean and cereal crops in potato fields in East Africa reduced *R. solanacearum* to less than 50% compared to the controlled monocrop which was higher than 80% (Lemaga et al., 2005).

Any contaminated irrigation water and wastewater from potato processing facilities must be disinfected. Adding 100 ppm of hydrogen peroxide to irrigation water can be sufficient to eliminate *R. solanacearum* (van Bueningen et al., 2005). A similar study conducted by Yao et al. (2010) using 1.3 ppm chlorine dioxide obtained >99% efficacy in inhibiting growth of *R. solanacearum*.

Other management techniques that can be used to prevent introduction and spread of *R. solanacearum* R3bv2 include:

- Plant healthy (certified) seed potatoes and propagative material in pathogen free soil (Janse, 2012; Jones et al., 2017).
- Use cover crops to reduce weeds and possibly nematodes (Momol et al., 2005).
- Use well-drained and leveled fields (Momol et al., 2005).
- **Important**: Use strict sanitation practices with equipment, tools, transplanting, irrigation water, storage facilities, packing materials, etc. (Janse, 2012; Momol et al., 2005) (see Survey Preparation, Sanitization and Clean-Up).
- Plow under crop residue immediately following harvest (Momol et al., 2005).
- Store and grade potatoes in the original place of production to avoid spreading *R. solanacearum* R3bv2 (Janse, 2012).
- Avoid use of ebb-and-flow and flooding irrigation systems in greenhouses (Jones et al., 2017).

Chapter

6

Research Needs

New technology, research or assessment is needed to:

- Improve the sensitivity and reliability of detection methods for R3bv2, especially methods that can distinguish R3bv2 strains from other *R. solanacearum* strains.
- Develop resistant plant varieties that are commercially viable.
- Develop and/or screen additional chemical control products.
- Study the effects of cover crops, crop rotation and mulches on pathogen dynamics and disease incidence.
- Determine which chloropicrin treatment provides better results.

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Appendix

A

Environmental Compliance

Introduction

Use *Environmental Compliance* as a guide to environmental regulations pertinent to *Ralstonia solanacearum* R3bv2.

Overview

Program managers of Federal emergency response or domestic pest control programs must ensure that their programs comply with all Federal Acts and Executive Orders pertaining to the environment, as applicable. Two primary Federal Acts, the National Environmental Policy Act (NEPA) and the Endangered Species Act (ESA), often require the development of significant documentation before program actions may commence. Environmental and Risk Analysis Services (ERAS), a unit of APHIS' Policy and Program Development Staff (PPD), is available to provide guidance and advice to program managers and prepare drafts of applicable environmental documentation. In preparing draft NEPA documentation ERAS may also perform and incorporate assessments that pertain to other Acts and Executive Orders, described below, as part of the NEPA process. The Environmental Compliance Team (ECT), a part of PPQ's Emergency Domestic Programs (EDP), assists ERAS in development of documents and implements any environmental monitoring. Program leadership is strongly advised to consult with ERAS and/or ECT early in the development of a program in order to conduct a preliminary review of applicable environmental statutes and to ensure timely compliance.

Environmental monitoring of APHIS pest control activities may be required as part of compliance with environmental statutes, as requested by program managers, or as suggested to address concerns with controversial activities. Monitoring may be conducted with regards to worker exposure, pesticide quality assurance and control, off-site chemical deposition, or program efficacy. Different tools and techniques are used depending on the monitoring goals and control techniques used in the program. Staff from ECT will work with the program manager to develop an environmental monitoring plan, conduct training to implement the plan, provide day-to-day guidance on monitoring, and provide an

interpretive report of monitoring activities.

The following is list of pertinent laws and Executive Orders:

National Environmental Policy Act (NEPA) – NEPA requires all Federal agencies to examine whether their actions may significantly affect the quality of the human environment. The purpose of NEPA is to inform the decision-maker prior to taking action and to inform the public of the decision. Actions that are excluded from this examination, actions that normally require an Environmental Assessment, and actions that normally require Environmental Impact Statements are codified in APHIS' NEPA Implementing Procedures located in 7 CFR 372.5.

The three types of NEPA documentation are:

1. **Categorical Exclusion**
   Categorical exclusions are classes of actions that do not have a significant effect on the quality of the human environment and for which neither an environmental assessment (EA) nor an environmental impact statement (EIS) is required. Generally, the means through which adverse environmental impacts may be avoided or minimized have actually been built into the actions themselves (see 7 CFR 372.5(c)).

2. **Environmental Assessment (EA)**
   An EA is a public document that succinctly presents information and analysis for the decision-maker of the proposed action. An EA can lead to the preparation of an environmental impact statement (EIS), a finding of no significant impact (FONSI), or the abandonment of a proposed action.

3. **Environmental Impact Statement (EIS)**
   In the event that a major Federal action may significantly affect the quality of the human environment (adverse or beneficial), or, the proposed action may result in public controversy, an EIS is prepared.

Endangered Species Act (ESA) – This statute requires that programs consider their potential effects on federally protected species. The ESA requires programs to identify protected species and their habitat in or near program areas and documentation of how adverse effects to these species will be avoided. The documentation may require review and approval by the U.S. Fish and Wildlife Service and the National Marine Fisheries Service before program activities can begin. Knowingly violating this law can lead to criminal charges against individual staff members and program managers.

Migratory Bird Treaty Act – This statute requires that programs avoid harm to

over 800 endemic bird species, eggs, and their nests. In some cases, permits may be available to capture birds, which require coordination with the U.S. Fish and Wildlife Service.

Clean Water Act – This statute requires various permits for work in wetlands and for potential discharges of program chemicals into water. This may require coordination with the Environmental Protection Agency, individual states, and the Army Corps of Engineers. Such permits would be required even if the pesticide label allows for direct application to water.

Tribal consultation – This Executive Order requires formal government to government communication and interaction if a program might have substantial direct effects on any federally-recognized Indian Nation. This process is often incorrectly included as part of the NEPA process, but must be completed prior to general public involvement under NEPA. Staff should be cognizant of the conflict that could arise when proposed federal actions intersect with tribal sovereignty. Tribal consultation is designed to identify and avoid such potential conflict.

National Historic Preservation Act – This statute requires programs to consider potential impacts on historic properties (such as buildings and archaeological sites) and requires coordination with local State Historic Preservation Offices. Documentation under this act involves inventorying the project area for historic properties and determining what effects, if any, the project may have on historic properties. This process may require public involvement and comment prior to the start of program activities.

Coastal Zone Management Act – This statute requires coordination with states where programs may impact Coastal Zone Management Plans. Federal activities that may affect coastal resources are evaluated through a process called “federal consistency”. This process allows the public, local governments, Tribes, and state agencies an opportunity to review the federal action. The federal consistency process is administered individually by states with Coastal Zone Management Plans.

Environmental Justice – This Executive Order requires consideration of program impacts on minority and economically disadvantaged populations. Compliance is usually achieved within the NEPA documentation for a project. Programs are required to given consider if the actions might disproportionally impact minority or economically disadvantaged populations, and if so, how such impact will be avoided.

Protection of Children –This Executive Order requires federal agencies to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. If such a risk is identified, then measures must

be described and implemented to minimize such risks.

Appendix

B

USDA–APHIS Procedures for Submitting Samples

Symptomatic, i.e., wilting, plants can be tested for the presence of *Ralstonia solanacearum* bacteria using immunostrips available from commercial providers listed below, or by ELISA testing. Positive reactions will only indicate the presence of *R. solanacearum*, but not what race is present. *R. solanacearum* R3bv2 is considered a federal select agent and is not known to occur in the United States. *R. solanacearum* race 1 is considered endemic to the southern United States and is not a federal quarantine pest or select agent.

The following commercially available immunostrips are approved by APHIS for field level screening:

1. https://orders.agdia.com/agdia-immunostrip-for-rs-isk-33900, AgDia, Inc. Elkhart, IN
2. Potato Brown Rot Pocket TM Pocket Diagnostic, from Forsite Diagnostics Ltd, York, UK

Because of changes in the Federal Select Agent Program (FSAP) regulations, all races and biovars are considered select agents until proven otherwise. Once a sample is determined to be positive for *R. solanacearum*, the sample must be handled according to FSAP's regulations. There is a form from the Division of Agricultural Select Agents and Toxins (DASAT), which is necessary to use depending on your course of action to A) destroy the sample or B) transfer the sample to a registered entity for further testing. The appropriate form needs to be completed and approved by DASAT. See below which provides the specific requirements on how to handle *R. solanacearum* positive samples and forms.

Here are links to the forms: **APHIS/CDC Form 4a** info is here for Reporting the Identification of a Select Agent or Toxin from a Clinical/Diagnostic Specimen and Guidance on How to Complete APHIS/CDC Form 4:

https://www.selectagents.gov/form4.html

This form should be completed by the appropriate persons and sent to the following e-mail address: DASAT@usda.gov or according to the instructions on the form and the guidance below.

Questions about the Select Agent Program should be directed to the above e-mail address or call: 301-851-2070.

There are two choices for plant dispositions that show positive for *R. solanacearum* using the immunostrip or ELISA: A) destroy the sample, or B) send for further testing at the USDA–APHIS–PPQ–S&T–Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL, formerly the Beltsville Lab). Below are the criteria to assist in those choices:

A. The sample may be destroyed according to the select agent regulations if they are not from a foreign source, are not unusual hosts, do not show unusual symptomatic reactions, or are from areas in the U.S. where *R. solanacearum* is known to occur.

   - Send an APHIS/CDC Form 4a (Report of Identification) to the DASAT and the sample is destroyed by the diagnostic lab. No further action required.

B. Samples fitting the following criteria will require further testing at PPCDL, approved for confirmation and select agent handling:

   1) Positive testing (*R. solanacearum*) samples with a connection to a foreign source (i.e., imported plants)

      - Forward the sample(s) to the USDA–APHIS–PPQ– S&T–PPCDL to be tested for *R. solanacearum* R3bv2.

      - Refer to the "Shipping instructions for samples requiring further testing at an approved laboratory" section for instructions.

      - Follow the select agent regulations listed below.
        a. Contact PPCDL to inform them of the sample and receive guidance through the APHIS/CDC reporting process.
        b. Send an APHIS/CDC Form 4a (Report of Identification) to DASAT. DASAT approval with an approved Form 2 (Request to Transfer) from the receiving lab is required before sample shipping.
        c. PPCDL will initiate a Form 2 (Request to Transfer) Section 1. Once completed, Section 2 will be sent to the submitter by DASAT for completion. After the Form 4a (Report of Identification) and Form 2 (Request to Transfer) Sections 1 and 2 are approved, the sample may be shipped.
        d. If positive for R3bv2, PPCDL will submit an APHIS/CDC Form 4a (Report of Identification) to DASAT.

e. Sample destroyed at both diagnostic and confirmatory labs.
f. Sample retained:
    i. If registered for agent, notify the DASAT of retention and put in inventory.

2) For other domestic detections of *R. solanacearum*, forward samples to PPCDL from unusual hosts, unique symptomatic reactions, and/or from areas in the country where *R. solanacearum* is not known to occur. The usual cultivated hosts of *R. solanacearum* race 3 biovar 2 include eggplant, geranium, pepper, potato, and tomato.
    a. Refer to the "Shipping instructions for samples requiring further testing at an approved laboratory" section for instructions.
    b. Contact PPCDL to inform them of the sample and receive guidance through the APHIS/CDC reporting process.
    c. Send an APHIS/CDC Form 4a (Report of Identification) to DASAT. DASAT approval with an approved Form 2 (Request to Transfer) from the receiving lab is required before sample shipping.
    d. PPCDL will initiate a Form 2 (Request to Transfer) Section 1. Once completed, Section 2 will be sent to the submitter by DASAT for completion. After the Form 4a (Report of Identification) and in the Form 2 (Request to Transfer) sections 1 and 2 are approved, the sample may be shipped.
    e. If positive for R3bv2, PPCDL will submit an APHIS/CDC Form 4a (Report of Identification) to DASAT.
    f. Sample destroyed at both diagnostic and confirmatory labs.
    g. Sample retained:
        i. If registered for the agent, notify the DASAT of retention and put in inventory.

For samples being destroyed, meeting the stated criteria:

- If identified as *R. solanacearum*, and the plant is not associated with a foreign source, not an unusual host, and from an area of thecountry where the organism is known to occur, no further race/biovar determination is required at an approved lab.
    1. Complete an APHIS/CDC Form 4a (Report of Identification).
    2. Send Form 4a to DASAT by email, fax or mail as indicated on the form.
    3. Destroy the sample.
    4. No further action is required (destruction records for the pathogen must be kept and maintained for a minimum of three years).

Shipping instructions for samples requiring further testing at an approved laboratory:

- For a positive *R. solanacearum* sample that requires further testing to confirm if it is race 3, biovar 2, the step-by-step procedures are summarized below.
- If identified as *R. solanacearum*, and further race/biovar determination is necessary at a Federal Select Agent Program registered lab because the plant is associated with a foreign source, or an unusual host and or from an area in the country where the organism is not known to occur:
    1. Contact PPCDL at APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov to inform them of the sample and receive guidance on sample submission.
    2. Complete sections A & B of the APHIS/CDC Form 4a (Report of Identification) and send to DASAT@usda.gov.
    3. PPCDL will complete Form 2 (Request to Transfer) Section 1.
    4. Complete Form 2 (Request to Transfer) Section 2 and send to DASAT@usda.gov.
    5. Coordinate with PPQ to generate an Agriculture Risk Management (ARM) Diagnostic Request (DR) Routing Form.
    6. Complete a PPQ Form 391.
    7. Send the sample, a copy of the approved Form 2, ARM DR, and PPQ Form 391 in the shipment to PPCDL.
    8. PPCDL, will receive the sample, complete the APHIS/CDC Form 2 Section 3, and send the completed paperwork to DASAT.
    9. PPCDL, will then determine if it is *R. solanacearum* R3bv2. If positive for R3bv2, PPCDL, will complete the APHIS/CDC Form 4a (Report of Identification), and send the APHIS/CDC Form 4a to DASAT.
    10. PPCDL, will notify the Domestic Diagnostics Coordinator of the test outcome, who will then report the results according to the Pest Identification Notification Protocol.

Sample Preparation and Sending Procedures

When sending the sample, you can send the whole plant including roots, but they must be **free of soil**. If rinsing with water, ensure sample is blotted dry prior to packing. At a minimum, enclose 0.035 ounces of symptomatic stem/crown tissue. Include completed hard copies of the PPQ form 391, the ARM DR routing form, and the APHIS/CDC Form 2 in the shipping container. Be sure to include cool packs in a sturdy insulated container and send by overnight carrier (do not send on a Friday) to the following address:

Plant Pathogen Confirmatory Diagnostics Laboratory
Bldg. 580, BARC-East

9901 Powder Mill Road
Laurel, MD 20708
Phone: (301) 504-7100, VOIP: (301) 313-9200

Group E-mail Address:
APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov

Once packaged and sent, please send an e-mail message to the group e-mail address above with a pdf of the completed PPQ form 391 and the overnight carrier tracking number.

Results Reporting

Results will be reported through the PPQ National Identification Services Domestic Diagnostics Coordinator and the National Survey Coordinator, according to the Pest Identification Notification to the States protocol. Sample submitters and originating laboratories will receive results through their State Plant Regulatory Officials (SPROs) or State Plant Health Directors (SPHDs).

Stephen W. Bullington, Ph.D.
Domestic Diagnostic Coordinator
National Identification Services
USDA–APHIS–PPQ
4700 River Road, Unit 52
Rm. 4D-04.35
Riverdale, MD 20737
301-851-2153
stephen.w.bullington@usda.gov

Aimee Hyten
Plant Pathologist
Division of Agricultural Select Agents and Toxins
4700 River Rd. Unit 2
Riverdale, MD 20737
301-851-3300, option 3
DASAT@usda.gov

R. solanacearum R3bv2

Authors and Reviewers

Author

Jennifer C. Cook, Ph.D., NCSU–CIPM

Editor

Rosemary Hallberg, M.A., NCSU–CIPM

Reviewer

Maria Acevedo-Sanchez, USDA–APHIS–PPQ

Caitilyn Allen, Ph.D., University of Wisconsin-Madison

Damon Crook, Ph.D., USDA–APHIS–PPQ

David Bednar, Ph.D., USDA–APHIS–PPQ

Kathryn E. Bronsky, USDA–APHIS–PPQ

Peter Hertl, Ph.D., USDA–APHIS–PPQ

Julie F. Orr, USDA–APHIS–PPQ

Jaap van Kretschmar, Ph.D., NCSU–CIPM

Ricardo Valdez, USDA–APHIS–PPQ

Contributors

Global Pest and Disease Database Team