New Pest Response Guidelines

*Ralstonia solanacearum* (Smith, 1896)
Yabuuchi et al., 1996 “race 3 biovar 2”

Brown rot of potato
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CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife—if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.
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Plant Protection and Quarantine (PPQ) develops New Pest Response Guidelines (NPRGs) in preparation for potential future pest introductions.

**Note:** 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, to be used as a basis for developing a situation-specific response plan at the time a new pest is detected.
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 16 °C (60.8 °F), but symptoms are most likely at 24–35 °C (75.2–95 °F).
- Primarily spread through contaminated irrigation and surface runoff water, infested soil, tools and equipment.
- Major hosts are potato (_Solanum tuberosum_ L.), tomato (_Solanum lycopersicum_ L.) and geranium (_Pelargonium_ spp.).
- Many native plant and weed species, such as bittersweet nightshade (_Solanum dulcamara_ L.), 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 phytotype 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

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**Biology and Ecology**

**Life Cycle**

*Ralstonia solanacearum* R3bv2 is soil and waterborne and can survive for days to years in infected soil (at least 75 cm (28.53’’)) (Champoiseau et al., 2009; Graham et al., 1979), as well as surface irrigation water and weeds (Champoiseau and Momol, 2009). This bacterium thrives in the cool tropical highlands and in temperate zones. *Ralstonia solanacearum* R3bv2 is highly virulent at temperatures between 19 °C and 28 °C (66.2 °F and 82.4 °F) (Huerta et al., 2015). Virulence decreases with temperatures above 35 °C (95 °F) or below 16 °C (60.8 °F) (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 4 °C (39.2 °F). 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 asymptotically infected native plant and weed species (Jones et al., 2017). In Europe, numerous outbreaks of *R. solanacearum* R3bv2 have been attributed to bittersweet nightshade (*Solanum dulcamara* L.), 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

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus viridis</em> L.</td>
<td>green amaranth</td>
<td>Lin et al. (2015)</td>
</tr>
<tr>
<td><em>Capsicum annuum</em> L.</td>
<td>sweet pepper</td>
<td>Martin and French (1995)</td>
</tr>
<tr>
<td><em>Capsicum spp.</em></td>
<td>pepper</td>
<td>Lin et al. (2015)</td>
</tr>
<tr>
<td><em>Oxalis latifolia</em> Kunth</td>
<td>broadleaf wood-sorrel</td>
<td>Khoodoo et al. (2010)</td>
</tr>
<tr>
<td><em>Pelargonium × hortorum</em> L. H. Bailey</td>
<td>zonal geranium</td>
<td>Williamson et al. (2002)</td>
</tr>
<tr>
<td><em>Pelargonium spp.</em></td>
<td>geranium</td>
<td>Ozakman and Schaad (2003)</td>
</tr>
<tr>
<td><em>Pelargonium zonale</em> (L.) L’Hér.</td>
<td>horseshoe pelargonium</td>
<td>Janse et al. (2004)</td>
</tr>
<tr>
<td><em>Physalis angulata</em> L.</td>
<td>cut-leaf ground-cherry</td>
<td>Swanepoel (1992)</td>
</tr>
<tr>
<td><em>Portulaca oleracea</em> L.</td>
<td>common purslane</td>
<td>Lin et al. (2015)</td>
</tr>
<tr>
<td><em>Solanum betaceum</em> Cav. (=<em>Cyphomandra betacea</em> (Cav.) Sendtn.)</td>
<td>tree-tomato</td>
<td>Martin and Nydegger (1982)</td>
</tr>
<tr>
<td><em>Solanum lycopersicum</em> L.</td>
<td>tomato</td>
<td>Mahbou Somo Toukam et al. (2009)</td>
</tr>
<tr>
<td><em>Solanum melongena</em> L.</td>
<td>aubergine</td>
<td>Caffier and Hervé (1996)</td>
</tr>
<tr>
<td><em>Solanum nigrum</em> L.</td>
<td>black nightshade</td>
<td>Tomlinson and Guntber (1986)</td>
</tr>
<tr>
<td><em>Solanum pimpinellifolium</em> L. (=<em>Lycopersicon pimpinellifolium</em> (L.) Mill.)</td>
<td>currant tomato</td>
<td>Khoodoo et al. (2010)</td>
</tr>
<tr>
<td><em>Solanum spp.</em></td>
<td>potato</td>
<td>Allen et al. (2005)</td>
</tr>
<tr>
<td><em>Solanum tuberosum</em> L.</td>
<td>potato</td>
<td>Tahat and Sijam (2010)</td>
</tr>
<tr>
<td><em>Solanum tuberosum</em> L. subsp. andigenum* (Juz. &amp; Bukasov)</td>
<td>Andean potato</td>
<td>Ciampi and Sequeira (1980)</td>
</tr>
</tbody>
</table>
### Table 2-2 List of reported asymptomatic hosts of *R. solanacearum* R3bv2

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ageratum conyzoides</em> L.</td>
<td>goatweed</td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Amaranthus</em> spp.</td>
<td>pigweed</td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Bidens pilosa</em> L.</td>
<td>beggar-ticks</td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Dopatrum</em> sp.</td>
<td>dopatrium</td>
<td>Pradhanang and Momol (2001)</td>
</tr>
<tr>
<td><em>Drymaria cordata</em> (L.) Willd. ex Schult.</td>
<td>tropical chickweed</td>
<td>Pradhanang et al. (2000)</td>
</tr>
<tr>
<td><em>Galinsoga parviflora</em> Cav.</td>
<td>dumb-nettle</td>
<td>Pradhanang and Elphinstone (1996)</td>
</tr>
<tr>
<td><em>Leucas martinicensis</em> (Jacq.) W. T. Aiton</td>
<td>whitewort</td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Monochoria vaginalis</em> (Burm. f.) C. Presl ex Kunth</td>
<td>pickerel-weed</td>
<td>Pradhanang and Momol (2001)</td>
</tr>
<tr>
<td><em>Nicotiana glutinosa</em> L.</td>
<td></td>
<td>Pradhanang and Elphinstone (1996)</td>
</tr>
<tr>
<td><em>Oxalis latifolia</em> Kunth</td>
<td>broadleaf wood-sorrel</td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Persicaria capitata</em> (Buch.-Ham. ex D. Don) H. Gross (=<em>Polygonum capitatum</em> Buch.-Ham. ex D. Don)</td>
<td>Japanese knotweed</td>
<td>Pradhanang and Elphinstone (1996)</td>
</tr>
<tr>
<td><em>Persicaria nepalensis</em> (Meisn.) H. Gross (=<em>Polygonum nepalense</em> Meisn.)</td>
<td>Nepal knotweed</td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Rumex abyssinicus</em> Jacq.</td>
<td></td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Solanum dulcamara</em> L.</td>
<td>bittersweet nightshade</td>
<td>Wenneker et al. (1999)</td>
</tr>
<tr>
<td><em>Stellaria sennii</em> Chiov.</td>
<td></td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Spergula arvensis</em> L.</td>
<td>corn spurrey</td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Tagetes minuta</em> L.</td>
<td>Aztec marigold</td>
<td>Tusiime et al. (1998)</td>
</tr>
<tr>
<td><em>Urtica dioica</em> L.</td>
<td>stinging nettle</td>
<td>Wenneker et al. (1999)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brassica juncea</em> (L.) Czern.</td>
<td>brown mustard</td>
<td>Pradhanang et al. (2000)</td>
</tr>
<tr>
<td><em>Brassica oleracea</em> L.</td>
<td>cabbage</td>
<td>Álvarez et al. (2008)</td>
</tr>
<tr>
<td><em>Brassica</em> spp.</td>
<td>wild mustard</td>
<td>Álvarez et al. (2008)</td>
</tr>
<tr>
<td><em>Calibrachoa</em> sp.</td>
<td>calibrachoa</td>
<td>Jans et al. (2004)</td>
</tr>
<tr>
<td><em>Cerastium glomeratum</em> Thuill.</td>
<td>mouse-ear chickweed</td>
<td>Pradhanang et al. (2000)</td>
</tr>
<tr>
<td><em>Cichorium endivia</em> L.</td>
<td>endive</td>
<td>Álvarez et al. (2008)</td>
</tr>
<tr>
<td><em>Datura stramonium</em> L.</td>
<td>jimsonweed</td>
<td>Fernandez (1986)</td>
</tr>
<tr>
<td><em>Nicandra physalodes</em> (L.) Gaertn.</td>
<td>broadleaf-nightshade</td>
<td>Pradhanang et al. (2000)</td>
</tr>
<tr>
<td><em>Nicotiana rustica</em> L.</td>
<td>Aztec tobacco</td>
<td>Martin and French (1995)</td>
</tr>
<tr>
<td><em>Petunia × atkinsiana</em> (Sweet) D. Don ex W. H. Baxter (=<em>Petunia × hybrida</em> hort. ex</td>
<td>common garden petunia</td>
<td>Janse et al. (2004)</td>
</tr>
</tbody>
</table>
**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 asymptotically 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 (*Populus* spp.) for 17 days, oak (*Quercus* spp.) for 4 days and on high-density polyethylene in cold storage at 4 °C (39.2 °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

<table>
<thead>
<tr>
<th>Region</th>
<th>Country</th>
<th>References</th>
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<tbody>
<tr>
<td>Africa</td>
<td>Burundi</td>
<td>Autrique and Potts (1987)</td>
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<td></td>
<td>Cameroon</td>
<td>Mahbou Somo Toukam et al. (2009)</td>
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<td>Egypt</td>
<td>Farag et al. (1999)</td>
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<td></td>
<td>Ethiopia</td>
<td>Lemessa and Zeller (2007)</td>
</tr>
<tr>
<td></td>
<td>Kenya</td>
<td>Janse et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Malawi</td>
<td>Zayamba Kagona (2008)</td>
</tr>
<tr>
<td></td>
<td>Mauritius</td>
<td>Khooodoo et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Nigeria</td>
<td>Popoola et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>Reunion</td>
<td>Nicole et al. (1998)</td>
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<tr>
<td></td>
<td>Rwanda</td>
<td>Uwamahoro et al. (2018)</td>
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<td></td>
<td>South Africa</td>
<td>Cellier et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Tanzania</td>
<td>Mwankemwa (2015)</td>
</tr>
<tr>
<td></td>
<td>Uganda</td>
<td>(Allen et al., 2005)</td>
</tr>
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<td>Asia</td>
<td>Bangladesh</td>
<td>Chakraborty and Roy (2016)</td>
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<td></td>
<td>China</td>
<td>Wang et al. (2017)</td>
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<td>India</td>
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<td>Indonesia</td>
<td>Horita and Tsuchiya (2001)</td>
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<td>Iran</td>
<td>Izadiyan and Taghavi (2013)</td>
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<td></td>
<td>Japan</td>
<td>Horita and Tsuchiya (2001)</td>
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<td>Korea, Republic of</td>
<td>Jeong et al. (2007)</td>
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<td>Nepal</td>
<td>Pradhanang et al. (2000)</td>
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<tr>
<td></td>
<td>Pakistan</td>
<td>Begum (2011)</td>
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<td></td>
<td>Philippines</td>
<td>Natural et al. (2005)</td>
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<tr>
<td></td>
<td>Sri Lanka</td>
<td>EPPO (1998)</td>
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<td></td>
<td>Taiwan</td>
<td>Wu et al. (2011)</td>
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<td>Turkey</td>
<td>Ustun et al. (2008)</td>
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<td>Europe</td>
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<td></td>
<td>Georgia</td>
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<td></td>
<td>Greece²</td>
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<td></td>
<td>Portugal</td>
<td>Cruz et al. (2012)</td>
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<td></td>
<td>Russia</td>
<td>Matveeva et al. (2003)</td>
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<td></td>
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<td>EPPO (2004)</td>
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<td>Spain</td>
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</tr>
<tr>
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<tr>
<td>Sweden¹</td>
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<td>New Caledonia</td>
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<td>Papua New Guinea</td>
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<td>South America</td>
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<tr>
<td>Argentina</td>
<td>French (1988)</td>
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<tr>
<td>Bolivia</td>
<td>Castillo and Plata (2016)</td>
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<tr>
<td>Brazil</td>
<td>Almeida et al. (2003)</td>
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<td>Chile</td>
<td>Ciampi et al. (1997)</td>
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<td></td>
<td>van der Wolf et al. (2004)</td>
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<td>Colombia</td>
<td>Lebeau et al. (2011)</td>
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<td>Prior and Fegan (2005a)</td>
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<td>Costa Rica</td>
<td>Gabriel et al. (2006)</td>
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<td></td>
<td>Williamson et al. (2002)</td>
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<tr>
<td>French Guiana</td>
<td>Deberdt et al. (2014)</td>
<td></td>
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<tr>
<td>Guadeloupe</td>
<td>Prior and Steva (1990)</td>
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<tr>
<td>Guatemala</td>
<td>Sanchez Perez et al. (2008)</td>
<td></td>
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<tr>
<td>Peru</td>
<td>Gutarra et al. (2017)</td>
<td></td>
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<tr>
<td>Uruguay</td>
<td>Siri et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>Venezuela</td>
<td>Garcia et al. (1999)</td>
<td></td>
</tr>
</tbody>
</table>

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 (USDA–APHIS–PPQ–S&T Fort Collins Lab, 2018a)

Figure 2-2  Tomatoes harvested acres for sale in the continental U.S. in 2012 (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 (NCSU-CIPM, 2019)
Species Description/Morphology

*Ralstonia solanacearum* R3bv2 is a gram-negative, rod-shaped, motile, strictly aerobic bacterium that is 0.5–0.7 × 1.5–2.0 cm (0.2–0.28 × 0.59–0.79”) 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 (Champoiseau et al., 2009) (Fig. 3-1). 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 (Champoiseau et al., 2009) (Fig. 3-2). 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 (Champoiseau et al., 2009) (Fig. 3-3).
- 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 (Jones et al., 2017) (Fig. 3-4).
- 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 (Champoiseau et al., 2009) (Figs. 3-5 and 3-6).
- 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 (image credit Amilcar Sanchez Perez)
**Figure 3-2** Cut section of a tomato stem displaying vascular browning caused by infection with *R. solanacearum* (image credit 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 (image credit Caitilyn Allen, University of Wisconsin-Madison)
**Figure 3-4** Bacterial ooze on geranium caused by infection with *R. solanacearum* R3bv2 (Margery Daughtrey, Cornell University, Bugwood.org)

**Figure 3-5** Left: Chlorosis on geranium plants caused by infection with *R. solanacearum* R3bv2; Right: Healthy geranium (image credit Caitlyn Allen, University of Wisconsin-Madison)
Figure 3-6  Early wilting symptoms on geranium caused by infection with \(R.\ solanacearum\) R3bv2 (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).
- \(Xanthomonas\ hortorum\ pv. pelargonii\) (Brown 1923) Vauterin et al. 1995, the causal agent of bacterial blight of geranium, produces wilting symptoms similar to \(R.\ solanacearum\) R3bv2 (Champosiseau et al., 2010).
- \(Fusarium\ oxysporum\ f. sp. lycopersici\) (Sacc.) W.C. Snyder & H.N. Hansen, 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).
- \(Verticillium\ albo-atrum\) Reinke & Berthold and \(V.\ dahliae\) Kleb., 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. \(sepedonicus\) (Spieckermann & Kotthof,)
Davis et al., causal agent of potato ring rot, produces a vascular ring containing bacterial ooze and causes leaf wilting during the hottest period of the day that reverses at night (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).
Delimitation Area

The delimitation area may be influenced by factors known only at the time of introduction, including the initial detection area, occurrence of high-risk pathways, density and distribution of hosts near the initial detection area, wind direction and available surveillance resources at the time of introduction. Information from trace-back and trace-forward investigations, the extent of natural and artificial spread of the disease, and agency funding and logistics can determine the size of the delimitation area. The size of delimiting survey boundaries can range from a small production site to a broad political or geographical boundary.

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.

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 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 100 °C (212 °F).

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/m² (1.36 ft-lb/in²) 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)

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Use Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols (&gt;60% ethanol)</td>
<td>Greenhouse hard non-porous surfaces (i.e. floor, walls, benches, counter tops, buckets, coolers)</td>
</tr>
<tr>
<td>Chlorhexidine (0.5%-4.0%)</td>
<td>Shoe wash</td>
</tr>
<tr>
<td>Chloroxylenol (0.3%-3.75%)</td>
<td>Greenhouse hard non-porous surfaces (i.e. floor, walls, benches, counter tops, buckets, coolers)</td>
</tr>
<tr>
<td>Iodine and iodophors (7.5%-10.0% povidone-iodine)</td>
<td>Shoe wash</td>
</tr>
<tr>
<td>Quatemary ammonium</td>
<td></td>
</tr>
<tr>
<td>Triclosan (0.2%-2.0%)</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Use Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>Greenhouse hard non-porous surfaces (i.e. floor, walls, benches, counter tops, buckets, coolers)</td>
</tr>
<tr>
<td>Iodine</td>
<td>Shoe wash</td>
</tr>
<tr>
<td></td>
<td>Greenhouse hard non-porous surfaces (i.e. floor, walls, benches, counter tops, buckets, coolers)</td>
</tr>
<tr>
<td></td>
<td>Shoe wash</td>
</tr>
</tbody>
</table>
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 a ha (A) 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, 91.44 cm (36”), walk down every 6th row looking at all plants in adjacent rows up to 6.1 m (20’) out.
    - If they are tightly spaced, 30.48 cm (12”), do the same every 20th row.
  - Take random samples to represent the entire ha (A).
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 immunostrips available from commercial providers. Positive reactions will indicate the presence of *R. solanacearum*, but will not distinguish the R3bv2 subgroup (USDA-APHIS-PPQ, 2020). 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 1 gm (0.04 oz.) 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 15.56 ºC (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 15.56 °C (60 °F).

g. Prepare samples within 72 hrs 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 15.56 °C (60 °F).

   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 0.47 L (0.50 qt.).

   c. Collect water samples at a depth of 30.48 cm (12”).

   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 15 °C (59 °F) 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 chapter presents known control options available for this pest and summarizes how widely used they are in the U.S.

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* R3 bv 2. The efficacy and feasibility of each control option should depend on the pest situation at the time of detection. Factors including detection location (e.g., natural or urban environment, agricultural crops, greenhouses, orchards), area of spread, the climatic region, the time of year, the phenology of the host and current practices already in place contribute to determining whether a particular control option is appropriate.

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 2 m (6.6’) or more of soil (Ebbels, 2003).

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**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 50 ml (10”) 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 25 kg/ha (22.3 lb./ac) 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 50 g m$^{-2}$ (1.47 oz yd$^{-2}$) and covered with either a 0.04 mm polyethylene film (PE) or a 0.04 mm (0.002”) totally impermeable film (TIF), while methyl bromide was applied at a rate of 40 g m$^{-2}$ (1.18 oz yd$^{-2}$) 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 < 50% compared to the controlled monocrop which was > 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).
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
Allen, C. 2019. Fwd: Ralstonia solanacearum R3bv2 NPRG draft. Personal communication to Jennifer.C.Cook@aphis.usda.gov on 19 February 2019, from callen@wisc.edu.


Champoiseau, P. 2009. Ralstonia solanacearum race 3 biovar 2 (Phylotype II, sequevar 1). From the field to the lab: Towards accurate identification of a select agent pathogen. Second National


143.


Zayamba Kagona, J. D. 2008. The incidence of bacterial wilt (Ralstonia solanacearum) in informal potato planting material used by farmers in Dedza and Ntcheu districts of Malawi. Masters of Science, Norwegian University of Life Sciences, Ås, Norway.

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
The following is a 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 disproportionately 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.
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* R2bv2 is considered a federal quarantine pest 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.

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

2.  Potato Brown Rot Pocket TM Pocket Diagnostic, from Forsite Diagnostics Ltd, York, UK

Because of changes in the Federal Select Agent Services regulations, all races and biovars are select agents. Once a sample is determined to be positive for *R. solanacearum*, the sample must be handled according to the Select Agent Program regulations. There are Agriculture Select Agent Services (AgSAS) forms necessary depending on your course of action to A) destroy the sample or B) send it for further testing. The appropriate form(s) need to be completed and approved by the AgSAS. See below which provides the specific requirements for how you 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 and Guidance on How to Complete APHIS/CDC Form 4:

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

**APHIS/CDC Form 2** info is here to Request to Transfer Select Agents and Guidance on How to Complete APHIS/CDC Form 2:

https://www.selectagents.gov/form2.html

These forms should be completed by the appropriate persons and sent to the
following e-mail address: agsas@aphis.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: 301-851-3300 option 3.

There are two choices for plants disposition that show a positive *R. solanacearum* using the immunostrip or ELISA: A) destroy the sample, or B) send for further testing at the CPHST Beltsville Laboratory.

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 AgSAS and the sample is destroyed by the diagnostic lab. No further action required.

B. Samples fitting the following criteria will require further testing at the CPHST Beltsville Laboratory, 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–CPHST Beltsville Laboratory to be tested for *R. solanacearum* R3bv2.

- Follow the select agent regulations listed below.
  a. Send an APHIS/CDC Form 2 (Request to Transfer) to AgSAS and AgSAS approval is required before sample shipping.
  b. The confirmatory lab must submit an APHIS/CDC Form 4a (Report of Identification) to AgSAS.
  c. Sample destroyed at both diagnostic and confirmatory labs.
  d. Sample retained:
     i. If registered for agent, notify the AgSAS of retention and put in inventory.
     ii. If not registered, request a written authorization from AgSAS to exclude the sample from select agent regulations. This written authorization applies only to races 1 & 2.

2) For other domestic detections of *R. solanacearum*, forward samples
to the CPHST Beltsville Laboratory 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 geranium (*Pelargonium* sp.), tomato, potato, pepper, and eggplant.

a. Send an APHIS/CDC Form 2 (Request to Transfer) to AgSAS and AgSAS approval is required before sample shipping.

b. The confirmatory lab must submit an APHIS/CDC Form 4a (Report of Identification) to AgSAS.

c. Sample destroyed at both diagnostic and confirmatory labs.

d. Sample retained:
   i. If registered for agent, notify the AgSAS of retention and put in inventory
   ii. If not registered, request a written authorization from AgSAS to exclude the sample from select agent regulations. This written authorization applies only to races 1 & 2.

*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 the country 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 Ag Select Agent Services (AgSAS) by 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).

*For samples requiring further testing at an approved laboratory:*

If a positive *R. solanacearum* sample requires further testing to confirm if it is race 3, biovar 2 at an Ag Select Agent Program registered laboratory (CPHST Beltsville Lab) because the sample is connected to a foreign source or is an unusual host or from an area in the country where the organism is not known to occur; then once you have submitted the APHIS/CDC Form 4a Reporting the Identification of a Select Agent and the APHIS/CDC Form 2-Request to Transfer Select Agents, and only after you have received notification that the select agent transfer has been approved by the Ag Select Agent Services, at that point, send the sample to the CPHST Beltsville Laboratory at the address below. The step-by-step procedures are summarized below.
If identified as *R. solanacearum*, and further race/biovar determination is necessary at an Ag Select Agent Services 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. The diagnostic lab should complete sections C & D of the APHIS/CDC Form 4A (Report the Identification) and an APHIS/CDC Form 2 (Request to Transfer).
2. Send the APHIS/CDC Form 2 to the Ag Select Agent Services for approval of the transfer.
3. Once approved, the diagnostic lab will complete the APHIS/CDC Form 2 and notify the CPHST Beltsville Lab of the intended shipment and shipping information.
4. Send the sample and copy of the Form 2 in the shipment to the CPHST Beltsville Lab.
5. The CPHST Beltsville Lab will receive the sample, complete the APHIS/CDC Form 2 paperwork and send the completed paperwork to the Ag Select Agent Services and the diagnostic lab.
6. The CPHST Beltsville lab will then determine if it is *R. solanacearum* R3bv2.
7. The CPHST Beltsville lab will complete the APHIS/CDC Form 4a-Reporting of Identification of a Select Agent,
8. And send the APHIS/CDC Form 4a to Ag Select Agent Services,
9. The CPHST Beltsville Lab 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 washed free of soil. At a minimum, enclose 1 gram of symptomatic stem/crown tissue. Include completed hard copies of [PPQ form 391 in the shipping container](<https://www.aphis.usda.gov/animal/plants/pestident/ppl_gps/services/2023/391/english/WAY-391.pdf>) and APHIS/CDC Form 2. 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:

Sample Diagnostics USDA–APHIS–PPQ–CPHST
BARC–East, Bldg.
580 Powder Mill Road
Beltsville, MD 20705-2350
Phone: (301) 504-7100, VOIP: (301) 313-9200

Group E-mail Address:
APHIS-PPQCPhSTBeltsvilleSampleDiagnostics@aphis.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).

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Cover Image

Potato wilting due to infection with R. solanacearum R3bv2 (image credit Juan Herrera)