

***Ralstonia solanacearum* Race 3 Biovar 2**

Scientific Name

Ralstonia solanacearum (Smith, 1896) race 3 biovar 2 Yabuuchi et al. (1995)

Synonyms:

Bacillus solanacearum, *Burkholderia solanacearum*, *Phytomonas solanacearum*, *Pseudomonas solanacearum*, and *Xanthomonas solanacearum*

Common Name(s)

Bacteria wilt disease (pepper, potato, and tomato), potato brown rot, Southern bacterial wilt and Southern wilt (geranium)

Type of Pest

Bacterium

Taxonomic Position

Class: Betaproteobacteria, **Order:** Burkholderiales, **Family:** Ralstoniaceae

Reason for Inclusion in Manual

CAPS Target: AHP Prioritized Pest List – 2006 through 2016

Pest Description

Ralstonia solanacearum is a bacterium that is known to infect over 200 plant species and causes extensive crop losses in economically important crops such as tomato, potato, tobacco, ginger, and peanut. It has been found on all continents with the exception of Antarctica (Swanson et al., 2005, 2007). *R. solanacearum* is genetically diverse (Table 1) and is composed of five different biovars, four phylotypes, and five different races. Races differ in virulence, symptom expression, and host range, whereas biovars differ biochemically in their ability to oxidize various disaccharides and hexose alcohols (Fig. 1, Table 2). Along with distinction of races and biovars, *R. solanacearum* can be further divided into phylotypes, which are based on evolutionary relatedness amongst biovar, race, and place of origin. Race 1 Biovar 1 of *R. solanacearum* is a common endemic pest in southern United States. The other races and biovars are exotic to the United States. Of all the types/variants of *R. solanacearum*, the United States is most concerned with Race 3 Biovar 2 (herein

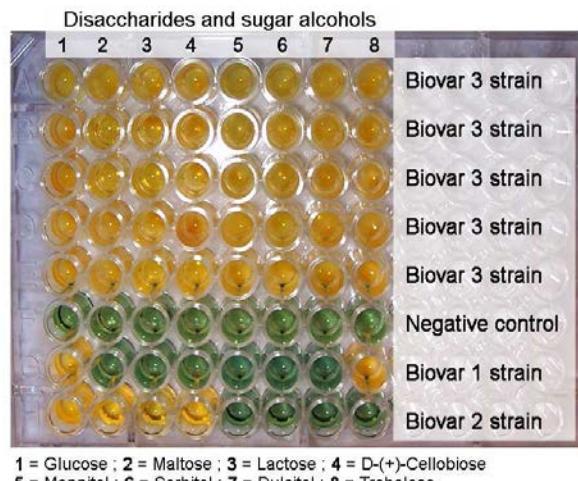


Figure 1. A microtiter plate showing the results of a biovar test with seven strains of *R. solanacearum*. Photo courtesy of Elizabeth Twieg, USDA-APHIS-PPQ-CPHST Beltsville Laboratory.

abbreviated R3 Bvr2). This particular race and biovar could be a major problem for the United States if it was introduced and established in terms of economic loss and trade restrictions (Floyd, 2008).

R. solanacearum R3 Bvr2, classified as phylotype II, is a quarantine pathogen in Europe and United States (Ozakman and Schaad, 2003). *R. solanacearum* R3 Bvr2 is thought to have originated from the Andean highlands in South America and has been able to move to other continents via potato tubers (Cook and Sequeria, 1991; Milling et al., 2009). This pathogen is now considered a major problem and is among the most destructive pathogens for potatoes (Swanson et al., 2005).

Table 1: Various types of *Ralstonia solanacearum* by race, biovar, geographic region and host range*

Race	Biovar	Distribution	Host
1	1, 3, 4	Asia, Africa, Australia, North America, and South America	Wide variety: Ginger, olive, chili pepper, peanut, <i>Solanum</i> spp., and tobacco
2	1	Caribbean, Asia, Central America, South America, and Hawaii	<i>Musa</i> spp. (banana, plantain), peanut, <i>Heliconia</i> , and tomato
3	2	Worldwide (except Canada and United States)	Solanaceous, and <i>Pelargonium</i> spp. (geranium)
4	4, 3	Australia, India, Asia, and Hawaii	Ginger
5	5	China	<i>Morus</i> spp. (mulberry)

*Summarized from Champoiseau, 2008; Janse et al., 2004; Ozakman and Schaad, 2003).

Table 2: Patterns of carbohydrate utilization / acid production for each of the five biovars of *Ralstonia solanacearum**.

Utilization of:	Biovars				
	1	2	3	4	5
Maltose	-	+	+	-	+
Lactose	-	+	+	-	+
Cellobiose	-	+	+	-	+
Mannitol	-	-	+	+	+
Sorbitol	-	-	+	+	-
Dulcitol	-	-	+	+	-

*Table reproduced from EPPO (2004)

Biology and Ecology

R. solanacearum R3 Bvr2 is a gram-negative soil and waterborne vascular pathogen (Wenneker et al., 1999; Swanson et al., 2007; Marco-Noales et al., 2008). *R. solanacearum* can live in the roots of host, in potato tubers, in the rhizosphere, in infected plant debris, and in non-hosts (Wenneker et al., 1999; Janse et al., 2004). *R. solanacearum* R3 Bvr2 can also survive in sterile water in a lab for many years (Milling et al., 2009). The bacterium is usually cultured on yeast extract-peptone-glucose agar (YPGA), non-selective media such as triphenyl terazolium chloride (TTC/TZC) agar and casamino acid peptone glucose (CPG), or semi-selective media (SMSA) between the temperatures of 28°C and 29°C (82.4°F and 84.2°F) (Cook and Sequeria, 1991; Caruso et al., 2003; Ozakman and Schaad, 2003; Marco-Noales et al., 2008).

Since the pathogen is able to survive in water and soil for a long period of time, *R. solanacearum* R3 Bvr2 can infect its host in many different ways. The pathogen can enter into the plant's xylem tissue through wounds leading to colonization, wilt and death (Milling et al., 2009). The pathogen can also spread in infected plant cuttings, seeds, and tubers. *R. solanacearum* is also able to invade the plant through young root hairs (Swanson et al., 2007). When infected plants decompose bacteria are released into the environment. Release can also occur through plant wounds, and in this case bacteria produce a "matrix of protective polysaccharides" that aids in survival (van Elsas et al., 2000). Once released, millions of bacterial cells can easily spread via irrigation water (Swanson et al., 2007).

Ralstonia solanacearum R3 Bvr2 is also known to be more cold tolerant than race 1 biovar 1 (R1 Bvr1), the native/endemic species to the United States. The cold resistance makes R3 Bvr2 a very worrisome pathogen for areas that grow solanaceous crops (tomato, potato, etc.). *R. solanacearum* R1 Bvr1 is primarily found in Southern United States (warm areas), but R3 Bvr2 may have a chance in surviving above and below the mid-Atlantic line (Swanson et al., 2005, 2007; Milling et al., 2009). In Australia, *R. solanacearum* R3 Bvr2 can survive in fallow soils with temperatures in the winter as low as 4°C (39.2°F) (Milling et al., 2009).

This pathogen can evade detection in symptomless hosts or latent hosts where symptoms are not expressed under unfavorable environmental conditions (Swanson et al., 2005). This characteristic can make it difficult to adequately survey for this pest. In Australia, Kenya, Sweden, and the United Kingdom under moderate conditions, this pathogen survives in deep soil and is known to live up to two years in soil where affected crops have already been removed (Wenneker et al., 1999; van Elsas et al., 2000). Little information on host resistance is known. Gorissen et al. (2004) found that use of pig waste and solarization can decrease population size and reduce survival of *R. solanacearum* R3 Bvr2 in soil.

Symptoms and Signs

Primary symptoms of bacterial wilt include: leaf chlorosis (yellowing), necrosis (browning) of vascular tissue, stunting, vascular rings, and rotting of tubers (Swanson et

al., 2005, 2007; Champoiseau et al., 2009). Wilted leaves are usually the first symptom observed followed by chlorosis and plant death (Champoiseau et al., 2009). The stem may collapse and gray-white bacterial ooze may be present on the stem, especially when the stem is cut or broken. Stems may also blacken (EPPO, 2004).



Figure 2: Generalized symptoms of *Ralstonia solanacearum* (all races/biovars) on tomato (left) and potato (right). **Upper Left:** Wilt symptoms on tomato. **Right:** Wilt symptoms on potato. **Bottom Left:** Vascular browning and brown streaks observed in a tomato stem. Photos courtesy of Clemson University - USDA Cooperative Extension Slide Series (left) and Central Science Laboratory, Harpenden Archive, British Crown (right). All photos are from www.bugwood.org.

On potatoes and other solanaceous hosts: The first visible symptom of *Ralstonia solanacearum* R3 Bvr2 on solanaceous hosts is wilting of the youngest leaves during the hottest part of the day, often on just one side of a leaflet or on a single branch. Another common symptom associated with bacterial wilt in the field is plant stunting. The entire plant may wilt quickly, leading to general wilting and yellowing of foliage and eventually plant death. A longitudinal slice of infected stems or stolons will reveal vascular browning, visible as long, narrow, dark brown streaks. In succulent young

plants of highly susceptible varieties, the stem can collapse, and gray-white bacterial ooze may be visible on stem surfaces (Figure 2).

In geranium: Symptoms of southern wilt on geranium can be subtle and easily overlooked. Symptoms usually begin with chlorosis and wilting of the lower leaves, then progress to an upward curling of leaf margins that is very characteristic. Under favorable conditions, the disease develops rapidly on geraniums and wilting may move upwards from older to younger leaves. Wilted leaves often develop wedge-shaped areas of chlorosis that become necrotic. The leaf margins may also become chlorotic, then necrotic, and the whole plant may desiccate and die. In late stages of disease, stems may collapse. Vascular discoloration is visible in stems (especially at the root crown) and roots; these can blacken and eventually become necrotic (Figure 3).



Figure 3: Left: geranium showing early wilting symptoms caused by *Ralstonia solanacearum* R3 Bvr2. Photo courtesy of Wisconsin Department of Agriculture, Trade and Consumer Protection. **Right:** A geranium showing yellowing symptoms caused by *Ralstonia solanacearum* R3 Bv2. Photo courtesy of Caitlyn Allen, University of Wisconsin-Madison.

In the early stages of disease in potato and geranium, the infected plants may appear to recover at night.

Pest Importance

According to Milling et al. (2009), about \$950 million is lost annually to *R. solanacearum* R3 Bvr2. *R. solanacearum* R3 Bvr2 was once found in a greenhouse in the United States on geranium plants, and that eradication effort alone cost the grower approximately \$10 million (Swanson et al., 2007).

Bacterial wilt is very difficult to control (Chamoiseau et al., 2009). *R. solanacearum* R3 Bvr2 is currently not present in Canada and United States. Its latent and cold tolerant features have made this pathogen even more difficult to control in areas where the pathogen is known to occur (Gorissen et al., 2004). Control measures are, therefore, aimed at preventing infection and establishment of the pathogen using exclusion.

Known Hosts

Major Hosts

Capsicum annuum (cayenne pepper), *Solanum esculentum* (tomato), *Solanum melongena* (eggplant), *Solanum* spp. (potato, tomato, etc...), and *Solanum tuberosum* (potato) (Ozakman and Schaad, 2003; Janse et al., 2004; Marco-Noales et al., 2008; Guidot et al., 2009; Milling et al., 2009).

Minor Hosts

Cyphomandra betaceae (tamarillo), *Pelargonium hortorum* (zonal geranium), *Pelargonium* spp. (geranium), *Pelargonium zonale* (horseshoe geranium), *Physalis angulata* (cutleaf groundcherry), and *Portulaca oleracea* (little hogweed) (Martin and Nydegger, 1982; Swanepoel, 1992; Ozakman and Schaad, 2003; Janse et al., 2004; Swanson et al., 2005).

The following solanaceous weeds are also considered hosts: *Solanum cinereum* (Narrawa burr), *Solanum dulcamara* (climbing nightshade), *Solanum nigrum* (black nightshade), and *Urtica dioica* (stinging nettle) (Wenneker et al., 1999).

Latent (symptomless) Hosts

Amaranthus spp. (pigweed/amaranth), *Bidens pilosa* (hairy beggarticks), *Cerastium glomeratum* (sticky chickweed), *Drymaria cordata* (whitesnow), *Eleusine coracana* (finger millet), *Galinsoga parviflora* (gallant soldier), *Galinsoga ciliata* (hairy galinsoga), *Oxalis latifolia* (broadleaf woodsorrel), *Polygonum capitatum* (pinkhead smartweed), *Rumex abyssinicum*, *Spergula arvensis* (corn spurry), *Stellaria sennii*, and *Tagetes minuta* (muster John Henry) (Wenneker et al., 1999; Pradhanang et al., 2000).

Experimental Hosts

Brassica juncea (brown mustard), *Eupatorium cannabinum* (hemp agrimony), *Ranunculus sceleratus* (cursed buttercup), *Stellaria media* (common chickweed), *Solanum phureja* (nightshade), *Tropaeolum majus* (nasturtium), and *Tussilago farfara* (coltsfoot) (Ciampi and Sequeria, 1980; Wenneker et al., 1999; Pradhanang et al., 2000).



Figure 4: Shows positive results for bacterial streaming. Photo Courtesy of David B. Langston, University of Georgia, www.bugwood.org.

Known Vectors or Associated Insects

It is possible that insects vector this bacterium as has been observed with other bacterial pathogens, but no specific vector has been identified at this time.

Known Distribution

R. solanacearum R3 Bvr2 has a worldwide distribution and is found in both temperate and tropical climates (Swanson et al., 2005).

Africa: Burundi, Cameroon, Egypt, Ethiopia, Kenya, Libya, Réunion Island, South Africa, and Zambia. **Asia:** Bangladesh, China, India, Indonesia, Iran, Japan, Java, Korea, Lebanon, Nepal, Pakistan, Philippines, Sri Lanka, and Taiwan. **Central America:** Costa Rica, Guadeloupe, Guatemala, and Peru. **Europe:** Belgium, France, Germany, Greece, Hungary, Netherlands, Russia, Serbia, Slovakia, Slovenia, Spain, Turkey and United Kingdom. **North America:** Mexico. **Oceania:** Australia and New Guinea. **South America:** Argentina, Bolivia, Brazil, Chile, Colombia, Peru, Uruguay, and Venezuela (Cook and Sequeria, 1991; Ozakman and Schaad, 2003; Janse et al., 2004; CABI, 2011; Guidot et al., 2009; Milling et al., 2009; Stefani et al., 2005; Swanson et al., 2007).

Nurseries/Greenhouse:

Europe: Belgium, Germany, and the Netherlands. **North America:** Canada and United States (eradicated) (Janse et al., 2004; Swanson et al., 2007; Marco-Noales et al., 2008).

Ralstonia solanacearum was detected in Austria in 2008, but it was declared eradicated in 2015 (EPPO, 2015).

Pathway

R. solanacearum R3 Bvr2 can be spread in contaminated irrigation, runoff, surface water, equipment, and soil (Wenneker et al., 1999; Caruso et al., 2003; Janse et al., 2004; Milling et al., 2009). The pathogen can be readily disseminated through infected propagative material, including potato tubers (Wenneker et al., 1999) and geranium cuttings (Milling et al., 2009). Plants may be infected but remain symptomless and shed large numbers of bacteria through their roots, making runoff water an important source of infection. In 2003, infected geranium cuttings were shown to be responsible for introduction of *R. solanacearum* R3 Bvr2 in the United States, but this introduction was subsequently eradicated (Champosieu et al., 2010).

Potential Distribution within the United States

Introductions of *R. solanacearum* R3 Bvr2 in greenhouse geraniums occurred in several states in 2003, introduced from Kenya, and 2004, introduced from Guatemala. Both introductions were successfully eradicated (Williamson et al., 2002). Solanaceous crop growing areas throughout the United States are at considerable risk due to the cold tolerance of *R. solanacearum* R3 Bvr2.

Survey

CAPS-Approved Method*: Visual survey is the approved survey method for *R. solanacearum* R3 Bvr2. For visual survey, collect symptomatic plant material.

Survey Instruction Details:

Bacterial Streaming: A quick and easy test to diagnose bacterial wilt or brown rot is by putting petioles or stem in a tube of sterile water, if a bacteria cloud appears within 20 minutes, it is possible the plant is infected. This procedure only indicates that wilt may

be caused by a bacterial pathogen and may not be effective in early stages (Champosieu et al., 2010) (Figure 4).

In Geranium: Collect stems from symptomatic plants (wilting; yellowing of lower leaves). Check for bacterial exudation by placing a piece of stem from a symptomatic plant into water, look for viscous streaming.

In Potatoes: Slice tubers and look for ooze, vascular discoloration (can incubate at 30°C for 3-to-4 weeks) (Figure 5).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at <http://caps.ceris.purdue.edu/>.

Literature-Based Methods:

Water sampling: Sampling for *R. solanacearum* from natural bodies of water is more reliable when the water temperature is above 15°C (59°F). In order to increase the likelihood for detection, each site should be sampled at several time points to reduce the effects of environmental variation (Momol et al., 2006).

- 1) Disposable sterile tubes should be used to collect the water at a depth of 12 to 16 inches (30 to 40 cm) and at least 6.6 feet (2 meters) from the bank. The tube should be filled after it has reached the sampling depth. Only use shatter proof tubes; do not use glass tubes.
- 2) At a given site, sampling should occur at three places with two replicas each (six samples total) at 9.8 feet (3 meter) intervals.
- 3) Label each tube with the sample location, date, and sample identification number.
- 4) Survey each sample location a minimum of four times during the season.
- 5) Store samples in the dark between 4°C and 10°C (39 and 50°F). Samples should arrive at the testing facility within 24 hours (Momol et al., 2006).



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Figure 5: A potato tuber that shows symptoms of slime ooze and browning of the vascular rings caused by *Ralstonia solanacearum*. Photo Courtesy of Plant Protection Service Archive, Plant Protection Service, www.bugwood.org.

Key Diagnostics/Identification

CAPS-Approved Method*:

Methods to confirm *Ralstonia solanacearum*: States can identify to genus and species by one of these methods, but race/biovar testing and confirmation is required at the CPHST-Beltsville Laboratory:

1. **Serological:** Test kits/ELISA kits can be used to identify to genus and species. Commercially available in the form of Immunostrips from Agdia and Lateral Flow Devices (Pocket Diagnostics) from Fera (formerly CSL).
2. **Culture:** To identify to genus and species.
 - a. SMSA semi-selective medium at 28 to 30°C (82 to 86°F) for 48 hours; *R. solanacearum* appears mucoid with a whitish colony.
 - b. TZC selective medium at 28 to 30°C (82 to 86°F) for 36 to 48 hours; *R. solanacearum* appears as mucoid, whitish colonies that produce pink pigmented centers (that do not diffuse into the medium).
3. **Molecular:** Weller et al. (2000) developed a real-time, multiplex polymerase chain reaction (PCR) assay to detect all known strains of *R. solanacearum* and a specific assay for the biovar 2A genotype (both reactions in a single tube). Another multiplex reaction that incorporates a third primer set, with an internal control, was developed for detection of *R. solanacearum* in infected potato tissue.

PPQ-CPHST has validated work instructions available upon request for:

- a. Conventional PCR detection of *R. solanacearum* and biovar 2 (Fegan et al., 1998).
- b. Real-time PCR that is a modification of the real-time PCR assay provided from CSL based on Weller et al. (2000).

For race and biovar testing: Once *Ralstonia solanacearum* has been confirmed, samples or cultures are sent to CPHST- Beltsville laboratory for race and biovar confirmation.

Biovar Plate Assay: A plate assay that differentiates biovar based on pure culture utilization of carbohydrates and sugar alcohol utilization is available. A work instruction from PPQ-CPHST is available.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at <http://caps.ceris.purdue.edu/>.

Literature-Based Methods:

Tomato bioassay: Using 2-to 3-week-old tomato seedlings to display symptoms. Wilting usually develops within a 1- to 3-weeks of inoculation when inoculated in a greenhouse kept at 25 to 28°C (77 to 82°F) (Lin et al., 2008).

Twitching motility: It is helpful to observe the microscopic colonies at 24 to 36 hours on solidified rich growth media (*i.e.*, SMSA, TZC) for twitching motility. Twitching motility is a form of translocation of bacteria over solid surfaces involving Type IV pili system. Relatively few bacteria species will exhibit this distinctive colony morphology and those that do can be distinguished from *R. solanacearum* once visible colonies develop (Champosieu et al., 2010).

Molecular: The 8X draft sequence of UW551 (R3 Bvr2 strain) genome was published and led to the identification of R3 Bvr2-specific genes (Gabriel et al., 2006). This information will facilitate future Race 3 specific molecular diagnostic assay development (Champosieu et al., 2010).

DNA probe: Cook and Sequeira (1991) developed a DNA probe based test to identify *Pseudomonas solanacearum* race 3.

Real-time BIO-PCR: Ozakman and Schaad (2003) developed a real-time BIO-PCR (includes a 24-hr enrichment phase) to detect *R. solanacearum* R3 Bvr2 in asymptomatic potato tubers.

PCR: Opina et al. (1997) used PCR primers developed from cloned RAPD fragments to identify *Burkholderia solanacearum*. Fegan (1998) developed a PCR based assay to detect the *R. solanacearum* biovar 2. There was a little cross-reactivity with three isolates of biovar 1 from potato. Pastrik and Maiss (2000) developed a new PCR assay for detection of *R. solanacearum* in potato tubers. Ten different DNA extraction techniques were compared. Of those ten techniques, Easy-DNA, SDS, and CTAB were considered highly reliable.

Poussier and Luisetti (2000) developed a nested PCR-RFLP protocol that detected biovars of *Ralstonia solanacearum* in plant tissue. A PVPP/BSA treatment is advised prior to PCR, because some plant compounds inhibited amplification.

Pradhanang et al. (2000) compared detection methods in soil, including nested PCR and conventional PCR. Both methods worked better when first enriching the infested soil sample in SMSA broth.

Glick et al. (2002) developed a multiplex PCR that could simultaneously detect *R. solanacearum* and *Xanthomonas campestris* pv. *pelargonii*, the two major bacterial pathogens of geranium.

Schönfeld et al. (2003) developed a PCR assay based on *fliC* fragments to detect *R. solanacearum* in soil.

Co-operational PCR (Co PCR): Caruso et al. (2003) developed a Co PCR reaction (based on the simultaneous and co-operative action of three primers) for detection of *R. solanacearum* in water. It can detect biovars 1, 2 and 4. This method was utilized by Marco-Noales et al. (2008) to detect non-culturable cells of *R. solanacearum* in asymptomatic geranium.

Loop-mediated amplification (LAMP) technique: Kubota et al. (2008) were able to detect *R. solanacearum* using this technique and a set of four primers and indicated it may be useful for identifying *R. solanacearum* from the soil.

Immunocapture and PCR (IC-PCR): Dittapongpitch and Surat (2003) combined immunocapture and PCR to detect *R. solanacearum* in soil and weeds. Soil detection required an enrichment step in nutrient broth.

Multiple Method Evaluation: Elphinstone et al. (1996) examined the sensitivity of various *R. solanacearum* detection methods when using potato tuber extracts. Culture on semi-selective media, ELISA, indirect immunofluorescent-antibody staining (IFAS) of fixed cells, immunofluorescent colony staining (IFCS), detection of specific DNA sequences following amplification by PCR, and bioassay in tomato seedlings were evaluated. Both ELISA and PCR methods were improved by pre-enrichment of samples in semi-selective broth prior to testing. A nested PCR method could detect fewer than 10 cells per ml in the potato extracts. Of other methods only dilution plating on semi-selective medium and tomato bioassay could detect fewer than 10 cells per ml. In order to combine ease and speed and use with sensitive detection, it was recommended that a series of methods be used for routine screening of potato tuber stocks for infection by *R. solanacearum* in Europe.

Easily Confused Species

R. solanacearum R3 Bvr2 is sometimes confused with *Xanthomonas campestris* pv. *pelargonii* in geranium. *Clavibacter michiganensis* subsp. *sepedonicus* produces vascular browning in potato as well (Champosieau et al., 2010). *R. solanacearum* R3 Bvr2 can also be confused with other races and biovars of *R. solanacearum*, *Erwinia carotovora* pathovars, *Fusarium* spp., and *Verticillium* spp. (Cook and Sequeria, 1991).

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Update History:

April, 2015: Updated to include the eradication from Austria.