Pest Data Sheet
Ralstonia solanacearum race 3 biovar 2

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PEST DATA SHEET
Ralstonia solanacearum* Race 3 Biovar 2 (Smith) Yabuuchi *et al.

TAXONOMIC POSITION

Kingdom: Proteobacteria
Class: Neisseriae
Order: Burkholderiales
Family: Burkholderiaceae

HOSTS

Primary hosts: *Solanum tuberosum* (potato) (CABI, 2001), *Lycopersicon esculentum* (tomato) (CABI, 2001)
Other solanaceous plants: *S. melongena* (eggplant) (NPAG, 2001b), *S. nigrum* (black nightshade) (NPAG, 2001b), *S. dulcamara* (bittersweet or climbing nightshade) (PLANTS Database), and *Datura stramonium* (NPAG, 2001b).
Other non-solanaceous hosts include: *Brassica* spp. (Janse *et al.*, 2002), *Cerastium glomeratum* (Pradhanang *et al.*, 2000), *Chenopodium album* (Janse *et al.*, 2002), *Drymaria cordata* (Pradhanang *et al.*, 2000), *Melampodium perfoliatum* (NPAG, 2001b), *Pelargonium hortorum* (geranium) (NPAG, 2001b), *Polygonum capitatum* (Pradhanang *et al.*, 2000), *Portulaca oleracea* (NPAG, 2001b), *Stellaria media* (Pradhanang *et al.*, 2000), *Tropaeolum majus* (Janse *et al.*, 2002), *Urtica dioica* (PLANTS Database; Wenneker *et al.*, 1999).
Wild hosts: Solanaceae (CABI, 2001)

DISTRIBUTION

Worldwide:
EPPO Region: Belgium, Germany, Hungary, Netherlands, Spain, Canary Islands, United Kingdom, England, Lebanon (CABI/EPPO, 1999)
Asia: Bangladesh, China (Fujian, Guangdong Guangxi, Hebei, Jiangsu, Taiwan, Zhejiang), India (Himachal Pradesh, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal), Indonesia (Java), Iran, Japan (Kyushu), Nepal, Pakistan, Philippines, Sri Lanka (CABI/EPPO, 1999)
Africa: Burundi, Egypt, Kenya, Libya, Réunion, South Africa, Zambia (CABI/EPPO, 1999)
South America: Argentina, Bolivia, Brazil (Goias, Parana, Pernambuco, Rio Grande do Sul, Santa Catarina, Sao Paulo), Chile, Colombia, Peru, Uruguay (CABI/EPPO, 1999)
Central America and Caribbean: Costa Rica, Guadeloupe, Mexico (CABI/EPPO, 1999)
Oceania: Australia (New South Wales, South Australia, Victoria) Papua New Guinea (CABI/EPPO, 1999)

BIOLOGY

Ralstonia solanacearum may have originated in the temperate highland regions of Peru and Bolivia (Van der Wolf and Perombelon, 1997). The term race is based on host range,

while biovar is based on biochemical tests. *Ralstonia solanacearum* race 3 biovar 2 is adapted to lower temperatures than what is found for other *R. solanacearum* races (Van der Wolf and Perombelon, 1997). It is a strictly aerobic, gram negative, non-spore forming, noncapsulated, nitrate-reducing, ammonia-forming, rod-shaped bacterium (Stevenson *et al.*, 2001). On tetrazolium chloride (TZC) medium it forms irregular shaped, fluidal, white with pink centered colonies (Stevenson *et al.*, 2001).

EPIDEMIOLOGY

Ralstonia solanacearum race 3 is a soilborne pathogen that persists in wet soils, deep soil layers (>75 cm), and reservoir plants (Van der Wolf and Perombelon, 1997). Its distribution in potato fields can be spotty, and is commonly found in areas that have poor drainage (Stevenson *et al.*, 2001). It is adapted to low temperatures, however its survival in very cold temperatures is reduced. In a study conducted in potato fields (Dirk van Elsas *et al.*, 2000), *R. solanacearum* race 3 biovar 2 population densities declined at 15 and 20 °C and was severely reduced at 4 °C. Severe drought negatively impacted population densities. Race 3 biovar 2 is most severe between 24-35 °C (optimal temperature of 27 °C) and decreases in virulence when temperatures exceed 35 °C or fall below 10 °C (Stansbury *et al.*, 2001). In regions such as Australia, England, Kenya, and Sweden the organism was not detected in previously diseased potato fields after two years, suggesting that long-term survival in temperate regions is reduced (Van der Wolf and Perombelon, 1997). In another study the bacterium persisted for 12 months in potato fields (Dirk van Elsas *et al.*, 2000). It is spread through infected potato tubers and can move plant-to-plant through the soil; it is not airborne (Stevenson *et al.*, 2001). The bacterium can survive in waterways and on weed hosts (Stevenson *et al.*, 2001). In geranium production, ebb and flow irrigation systems in greenhouses are conducive to disease (NAPPO, 2001).

DISEASE SYMPTOMS ON PRIMARY HOSTS

On Potato:

Initially, leaves wilt during the day, but recover during the nighttime (Smith *et al.*, 1997). Leaves may develop a bronze cast and petioles may develop epinasty (Smith *et al.*, 1997). Plants may become stunted and chlorotic. In the advanced stages of the disease, the lower stem will have a streaked brown appearance (Smith *et al.*, 1997). The vascular ring will be stained brown (DEFRA, 2001). If bacterial populations are high enough, a white mass of bacteria will ooze from a cut made in a symptomatic stem (Hay, 2001). Bacterial ooze may collect in the tuber eyes and soil may stick to secretions (Priou and Aley, 1999). Eventually, plants fail to recover and die (Stevenson *et al.*, 2001).

Diagnostics for potato:

Cross sections of the stem reveal brown discoloration of the vascular system (Stevenson *et al.*, 2001). Bacterial exudation may be observed by placing a piece of stem tissue from a symptomatic plant into a beaker of water and watching for viscous streaming (Smith *et al.*, 1997). Tubers can be screened by slicing tubers and looking for ooze, or if not immediately evident, incubate tubers for 3-4 weeks at 30 °C and observe for ooze from eyes. This method is time consuming and may not show low infection rates (Priou and Aley, 1999). The above procedures only confirm the presence of bacteria, but do not provide information on the exact genus/species nor biovar present. Other techniques that

do provide genus/species and depending on the assay, race and biovar information include: semi-selective media (Englebrecht, 1994); immuno-fluoresence staining (IF); enzyme-linked immuno-sorbent assay (ELISA) (Robinson-Smith *et al.*, 1995); molecular analysis using polymerase chain reaction (Seal *et al.*, 1993; Ozakman and Schaad, 2002); and pathogenicity testing.

On Geranium:
Plants develop a systemic wilt and initially recover during cooler temperatures or at night. The lower leaves tend to wilt first and become chlorotic (Douglas, 2002). V-shaped chlorotic or necrotic areas can develop on the leaves, as seen with bacterial blight caused by *Xanthomonas campestris* pv. *pelargonii* (Douglas, 2002). Brown discoloration of the vascular system is sometimes visible (Douglas, 2002). Eventually plants never recover from wilt and die (Douglas, 2002). Leaf spots are rarely present (Wick, 2001).

Diagnostics for Geranium:
Accurate detection at the ports of entry is difficult due to the lack of detection methods (Malik, 2002). Plants will develop a systemic wilt, but rarely will leaf spots be present (Wick, 2001). Hayward's carbohydrate utilization test successfully identified *R. solanacearum* race 3 biovar 2 isolated from geranium (Hudelson *et al.*, 2002). The following assays may be utilized: biology temperature studies, carbon compound utilization, bioassays, dilution plating on semi-selective media, fatty-acid analysis, immunfluorescence microscopy, enzyme-linked immunosorbent assays (ELISA) and polymerase chain reaction. It is suggested that several of the techniques be used to get reliable detection (Elphinstone *et al.*, 1997; Seal, 1997).

Detecting *Ralstonia solanacearum* race 3 biovar 2 with Real-Time PCR

Real-time polymerase chain reaction (real-time PCR) is a technique that enables the detection of the PCR product as it is accumulating. This is accomplished by using a fluorescently labeled probe that is dislodged by the exonuclease activity of the Taq polymerase. The amount of released fluorescence is proportional to the amount of amplified product. This technique eliminates the need to run gel electrophoresis and Southern blot assay, two steps required in classical PCR. Results from real-time PCR can be obtained within 1-3 hours, as opposed to 1-2 days for classical PCR and can be quantified.

Real-time PCR was developed for the identification of *R. solanacearum* race 3 biovar 2 (Schaad *et al.*, 2001). This technique was refined through the development of real-time Bio-PCR, leading to increased sensitivity and detection of *R. solanacearum* race 3 biovar 2 in latent infected potato tubers (Ozakman and Schaad, 2002). The real-time PCR assay was sensitive to race 3 biovar 2 (detecting as few as 20 cells/ml) and results were obtained in 1-2 hours. Bio-PCR is slightly different than real-time PCR in that there is an enrichment phase that lasts about 24 hours. On the second day, real-time PCR is run and results obtained. Bio-PCR is much more sensitive than the straightforward real-time PCR and is very useful for detecting latent infections. Both assays can be run

simultaneously since the real-time PCR takes less time than real-time Bio-PCR. If a positive reaction is obtained from the real-time PCR, then the Bio-PCR can be stopped.

PATHOGEN SPREAD

Potato

R. solanacearum race 3 biovar 2 is spread through infected seed potatoes and contaminated equipment (Hay, 2001). Insects may also serve as a vector, as seen with another bacterial pathogen, *Clavibacter michiganensis* subsp. *sepedonicus* (Stevenson *et al.*, 2001). Symptomless tubers can carry a latent infection (Anonymous, 1995; Stevenson *et al.*, 2001). Irrigation water also plays an important role in spread (Hay, 2001; NAPPO, 2001). In the early 1990's, potatoes and irrigation water in England tested positive for *Ralstonia* (Hay, 2001). In other European countries *Ralstonia* has been found in the water ways, infecting roots of *Solanum dulcamara* (Hay, 2001; NAPPO, 2001). This plant is considered to be a primary source of inoculum (Hay, 2001). Growers may pull *Ralstonia*-contaminated water from the waterways for irrigation, inoculating their potato/tomato crops (Hay, 2001). The source of bacteria in waterways may have come from waste generated by the processing of infected potatoes (Hay, 2001; NAPPO, 2001).

Geranium

Bacterial wilt has only been observed in ebb and flow geranium production (NAPPO, 2001). Spread occurs through water and through the shipment of infected cuttings (NAPPO, 2001; NPAG, 2001b).

CONTROL

Potato:

Chemical control through soil fumigation and antibiotics (streptomycin, ampicillin, tetracycline and penicillin) has shown little suppression of *Ralstonia solanacearum* (Murakoshi and Takahashi, 1984; Farag *et al.*, 1982). Currently, weed hosts, such as *S. dulcamara*, are removed from waterways in Europe by herbicide treatment in attempts to eradicate *Ralstonia*. This data is not yet available (Hay, 2001).

Crop rotation of 5-7 years with non-susceptible crops is recommended (Smith *et al.*, 1997). Altering soil pH depending on the time of year may also be effective. For potatoes, lowering the pH to 4-5 in the summer and raising it to 6 in autumn helped to eradicate the pathogen (Smith *et al.*, 1997). Screening tubers by plating on SMSA medium, ELISA, PCR, and IF will reduce the likelihood of planting diseased tubers in the field (Stevenson *et al.*, 2001). Apparently resistant plants are often latently infected with various strains of *R. solanacearum* which are additional means of dissemination of the pathogen (Stevenson *et al.*, 2001).

Geranium:

Sanitation is essential in the geranium industry to protect against vascular pathogens. Limited access to greenhouses is accomplished through a single entry with double doors. Production greenhouses have concrete or rock floors, raised benches and drip irrigation

(Klopmeyer, 2002). There is no water flow between plants. Water quality is ensured through different treatments (UV, chemical or heat treatment) and routine testing (Anonymous, 2002b). Wash basins with soap and water located at the entry way help prevent the movement of organisms in and out of the greenhouse structure. Workers are clothed with lab coats or aprons and wear latex gloves. Gloves are changed or disinfected between every plant (Klopmeyer, 2002). Knives and other tools are disinfested between plants either with quaternary ammonium, sodium hypochlorite or flame sterilization (Anonymous, 2002b; Klopmeyer, 2002). Foot baths at greenhouse entries are used to prevent organisms from being tracked in and out of the greenhouse. Soilless, sterilized media is used. Greenhouses are cleaned at the end of the season with hypochlorite or quaternary ammonium, and in some regions, with a formaldehyde drench (Anonymous, 2002b; Klopmeyer, 2002). Also, at the end of the season irrigation systems are cleaned with ammonium compounds, nitric acid or hypochlorite solution (Anonymous, 2002b). The plant material is culture indexed over 1-3 years under temperatures that favor expression of *R. solanacearum* (and *X. campestris* pv. *pelargonii*) symptoms. This ensures that stock is pathogen free. Throughout the production process, greenhouses are scouted and ELISA assays routinely run to ensure clean stock (Klopmeyer, 2002). Weed control programs are in place within and around greenhouses (Anonymous, 2002b).

ECONOMIC IMPACT

Yield is reduced through potato crop damage, resulting in economic losses (Hay, 2001).

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