Ralstonia solanacearum Race 3, biovar 2: A Potato Disease Becomes a Challenge for Geranium Growers

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Background

Race 3, biovar 2 of *Ralstonia solanacearum* (R3bv2) has earned its position as a Select Agent because it is the cause of a destructive disease of potatoes known as brown rot or Southern bacterial wilt. According to the latest terminology that is based on molecular sequence data, R3bv2 corresponds to phylotype II, sequevar 1 within *R. solanacearum*. Because this strain is adapted to cooler conditions and thus can cause disease in temperate climates, it has been problematic in potato growing areas in Europe, where it has become established along riversides in association with the weed, *Solanum dulcamara*. The range of *Ralstonia solanacearum* includes the southern United States, but at this time R3bv2 does not appear to be established in the United States. Control measures applied to this pathogen in other countries have included excluding seed imports from areas known to be affected, roguing out infected and volunteer potato plants, and control of the weed host, *S. dulcamara*. Exclusion of the pathogen is the goal for the United States.

Brown rot of potato © C. Allen, University of Wisconsin

Although most notorious for its effects on potatoes, R3bv2 also infects tomato, eggplant and *Pelargonium* species, in which it causes similar symptoms and leads to a disease often called Southern bacterial wilt. It may cause latent infections, presenting a diagnostic challenge. *Pelargoniums*, including the familiar *Pelargonium* x *hortorum*, the florist's geranium, are a major greenhouse crop in the United States. Geraniums finished for sale in United States greenhouses are today produced largely from unrooted cuttings imported from offshore, where cultural and economic conditions are optimal for the business of geranium propagation.

R. solanacearum R3bv2 is established in areas of the countries where geraniums are primarily propagated. Studies on culture collections have shown that R3bv2 was imported into the United States as early as 1995. In 2002 and 2003, R3bv2 was inadvertently imported into the United States and Canada within symptomless cuttings of geranium that originated in Kenya, Guatemala and Costa Rica. The disease was detected during the greenhouse production season, before outplanting. Detection triggered regulatory actions resulting in stop-sale orders placed upon greenhouse businesses, followed by destruction of shipments of plant material found or suspected to be contaminated with the pathogen. As a result of these economically-stressful events, the geranium propagation industry worked closely with USDA-APHIS and the Society of American Florists to establish mutually-agreeable protocols for the enhanced reliability of clean-stock geranium production in offshore facilities.

Symptomatic geranium infected with *R. solanacearum* (R3bv2) © C. Allen, University of Wisconsin

Offshore production of geranium cuttings. Photo A © Ball FloraPlant. Photo B & C © Goldsmith Seed Co.

Conclusion and Outlook

The outbreaks of *R. solanacearum* R3bv2 in the United States greenhouse industry resulted in the delivery of extensive plant pathology education programs from universities and government agencies and a stimulation of much-needed research. The importation of *R. solanacearum* R3bv2 has stimulated important research into the biology and epidemiology of the pathogen. The regulations regarding handling of a Select Agent require that research be conducted only in highly secure areas.

The flower production industry gained a better awareness of the role of quarantines in preventing the establishment of new pathogens in the United States. The threat was successfully eradicated and, as a result, an improved set of sanitation protocols is followed by all geranium propagators importing to the United States. The groundwork has also been laid for improved diagnostic capability and faster response to outbreaks via the National Plant Disease Diagnostic Network (NPDN).

Geraniums in growth chamber. © D. Norman, University of Florida

Diagnostic Techniques

The National Plant Disease Diagnostic Network Diagnostics Subcommittee has created Standard Operating Procedures (SOP) for diagnosticians to use as a reference. This guide also contains notification of results tree and contact information. The most recent version of the SOP can be found on the NPDN website at: http://www.npdn.org/DesktopDefault.aspx?tabindex=1&tabid=19.

Symptoms on geranium include wilting of lower leaves, in which diffuse-margined chlorotic patches will gradually become necrotic. Leaves sometimes turn upwards as they wilt. Vascular discoloration may be present in the stem, and roots may be browned. Photo on left C. Warfield, NC State Univ. Photo in center © D. Norman, Univ. of Florida

Symptoms of bacterial wilt (AKA brown rot) caused by *R. solanacearum* R3bv2 on potato include stunting and yellowing of leaves and vascular discoloration within stems and tubers. Infected plants may wilt, turn brown and die. Bacterial ooze is sometimes visible when cut tubers are squeezed. The disease is most easily confused with ring rot caused by *Clavibacter michiganensis* subsp. *sepedonicus*. © C. Allen

Suspending the cut end of an *R. solanacearum*-infected geranium stem in a beaker of water may generate copious streaming of bacteria from the xylem. © C. Warfield, N.C. State University

Greenhouse growers have been given training from Cooperative Extension educators to help them distinguish *R. solanacearum* from another more common vascular wilt pathogen, *Xanthomonas campestris* pv. *pelargonii* (Xcp). Both cause stunting and wilting of geraniums, but only the Xcp causes small, round leaf spots 1/64-1/16" in diameter. Both pathogens are serious threats to geranium production, but only the *Ralstonia* disease is under federal quarantine as a select agent. Enzyme-linked immunosorbent assay (ELISA) tests are marketed as immunostrips that distinguish the two diseases in cases in which wilting is the only symptom.

Cultural characteristics

R. solanacearum has very white, fluidal colonies that grow well on standard laboratory media such as King's B or potato dextrose agar. Tetrazolium chloride (in TZC medium) will highlight *R. solanacearum* colonies with a red pigmentation.

Plate with Ralstonia and inset of closeup of a few colonies, both on TZC media. Plate © D. Norman, University of Florida. Inset ©Walter Gutierrez, N.C. State University

Distinguishing Strains

After initial screening by ELISA, polymerase chain reaction (PCR) tests are generally used in the testing of plants suspected of having bacterial wilt due to R3bv2. © D. Norman, University of Florida

There are 5 different biovars of *R. solanacearum*, classified according to their ability to acidify a set of carbohydrates. This image shows the reaction that distinguishes Biovar 2: its profile shows acidification of cellobiose, lactose and maltose. © D. Norman, University of Florida

All shipments of geraniums produced offshore are now carefully barcoded. This facilitates trace-forward and trace-back activities in the event that disease-suspect geraniums are detected at any point in the production chain. Propagators also conduct regular tests using immunological methods to check for *R. solanacearum* in their offshore production facilities.

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