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Secretariat CFCS  
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CFCS Treasurer  
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## EXOTIC COOL TOLERANT BACTERIAL WILT STRAINS ENTERING NORTH AMERICA

D.J. Norman, A. M. Bocsanczy, A.A. Khan, and J.M.F. Yuen. Department of Plant Pathology, University of Florida, IFAS, Mid-Florida Research and Education Center, Apopka, Florida, USA

**ABSTRACT:** Bacterial wilt pathogen *Ralstonia solanacearum* is known to infect hundreds of plant species including economically important crops such as tomato, potato, pepper and banana. The disease is widespread in tropical and subtropical areas of the world and with the globalization of agriculture, plant pathogens are frequently moved into new environments. Bacterial wilt can be disseminated via a variety of hosts including high dollar value ornamentals such as geranium, anthurium, and pothos. Most bacterial wilt strains are limited in their range by temperature; however, a concern has always been that cool tolerant strains of *R. solanacearum* might be introduced and spread into potato and tomato production areas in North America. Due to their potential to cause major economic damage, cool tolerant Race 3 Biovar 2 (R3B2) *R. solanacearum* wilt strain has been designated as a Select Agent under the U.S. Agriculture Bioterrorism Protection Act of 2002. Using molecular techniques and environmental chamber studies, we have been able to identify additional Race and Biovar populations of *R. solanacearum* that are cool tolerant and have the ability infect tomato under cool temperature conditions. By comparing the proteomics profile of different strains of *R. solanacearum*, including R3B2 and other strains able infect plants at 18°C, we have identified a number of candidate proteins with functions related to pathogenicity at low temperatures. We are currently comparing total genome sequences of cool tolerant strains to identify regions that might contribute to virulence at low temperatures.

Keywords: bacterial wilt, proteomics, *Ralstonia solanacearum*.

### Introduction

Agricultural globalization moves plant pathogens into new environments. Many of these plant pathogens are endemic to the tropical, subtropical, or temperate regions of the world; therefore their relocation poses limited risk to agriculture or the environment. One significant exception, however, is Bacterial Wilt caused by *Ralstonia solanacearum* (Smith 1896) Yabuuchi et al. 1996 (synonym *Pseudomonas solanacearum*).

Bacterial wilt is a common bacterial disease in tropical and subtropical areas of the world. This pathogen is known to infect hundreds of plant species encompassing 44 families (Hayward, 1991). Extensive damage to high value crops of banana, potato, tomato, and pepper production in warm climate regions are well documented. Most of the research conducted on bacterial wilt to date has been in relation to large acreage agronomic crops (Swanson et al., 2005; Williamson et al., 2002). Bacterial wilt strains are generally limited in their range by temperature; however, a concern has always

been that additional cool tolerant strains of *R. solanacearum* might be introduced and spread into potato and tomato production areas.

Because of historical evidence of its ability to survive under cool climate conditions and its ability to devastate economically vital solanaceous crops such as tomato and potato, the Race 3, Biovar 2 strain (R3B2) of *R. solanacearum* has been designated as a “Select Agent” under the Agricultural Bioterrorism Protection Act of 2002.

Strict regulations have been implemented by APHIS/USDA to prohibit disease establishment. Historically bacterial wilt has been disseminated via banana and potato propagative material (Buddenhagen, 1961; Harrison, 1961; Thurston, 1963), however; bacterial wilt R3B2 strains occasionally enter the United States via imported ornamental propagative stock.

Pathosystems studies previously conducted on other bacterial and fungal populations indicated that pathogens and hosts often reach a stasis. Crops in confined areas such as a greenhouse or a farm may develop a tolerance or resistance to a local specific pathogen. A change in cultural or environmental conditions at this local level may cause disease to erupt, however; the primary concern is that infected propagative materials may appear asymptomatic. When these materials are transported across borders, disease outbreak may occur on a new susceptible host population.

Among potential ornamental hosts *R. solanacearum* has been described infecting anthurium, pothos, ornamental ginger, gerbera, zinnia, salvia, verbena, heliconia, sunflower, and geranium (Jones, 1993; Norman & Yuen, 1998; Norman & Yuen, 1999; Strider et al., 1981; Williamson et al., 2002). Over the past ten years there have been a number of accidental introductions of R3B2 strains into the US and Canada (Norman et al. 2009). In all cases, crop eradication has been effective and there has been no confirmed spread to date in the US and Canada.

## **Material and Methods**

Beginning in 2001, our research team at Mid-Florida Research and Education Center began a detailed study of Bacterial Wilt disease incited by *R. solanacearum*. The project objectives included developing disease control guidelines, defining population taxonomy, understanding virulence in cool weather conditions and examining host sensitivity and resistance.

A BSL-2 Quarantine Facility was established to satisfy requirements of the USA PATRIOT Act of 2001 and the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, which were enacted in the wake of the September 11, 2001 attacks and the subsequent 2001 anthrax attacks. The facility is funded through ornamental plant industry donations.

A total of 107 *R. solanacearum* strains belonging to diverse biovars and races have been collected over a 10-year period from imported propagative stock and compared with 32 previously characterized *R. solanacearum* strains. We compared populations of

*R. solanacearum* entering North America on ornamental crop material using repetitive PCR (rep-PCR). Using rep-PCR primers, *R. solanacearum* populations can be distinguished by biovar and, to a limited extent, country of origin and original host. We also used AFLP and sequence data (Cytocrome b561, Endoglucanase) to support our results. We started work on strains transported on nursery stock in cooperation with the University of Puerto Rico with funding by the USDA T-STAR program.

Host range and pathogenicity studies were done at different temperatures with new cool tolerant Biovar 1 strains of *R. solanacearum* introduced and established in the United States. In order to identify factors at molecular level that allow certain strains to infect under cool weather conditions, a comparative proteomics study of cool virulent and non-virulent strains was performed at two temperatures. Proteins were extracted, sequenced, and expression levels were compared between cool virulent and cool non-virulent strains (Bocsanczy et al., 2010). Both secreted and cell-associated proteins were extracted from *R. solanacearum* strains growing in the rhizosphere of tomato seedling at both 18° and 30° C. Two-dimensional protein gels were run with strains from each population. DIGE2 gels were used in the protein analysis to define up regulated or down regulated proteins under different weather conditions (cool 18°C or warm 30°C) during the infection process. Over 400 proteins were sequenced and identified.

Currently, sequencing of a cool virulent and a non-virulent at low temperature strains is in progress. Comparative genomics studies are expected to identify genes that may contribute to virulence at low temperature of the cool virulent strain.

Research has recently been initiated in order to identify resistance genes and understand resistance mechanisms present in some solanaceous hosts. This research may provide insight into the host disease resistance process and identify pathways for development of disease resistant ornamental and vegetable varieties.

## **Results and Discussion**

The potential movement of a systemic pathogen, such as *R. solanacearum*, in propagative material between countries has likely been underestimated. Our research has shown that bacterial wilt strains are frequently moved from country to country in propagative material. Whether strains become an established outbreak is dependent on environmental factors and on host availability. In many locations throughout the world sanitary cropping is difficult and agrochemicals are costly. Resistant cultivars may not be available. If propagative crops are not examined for pathogens or are not considered susceptible, potential for spread is even greater. When new undescribed pathotypes of *R. solanacearum* are introduced from propagative material into new environments, the impact to local crops is unpredictable.

Our rep-PCR evaluation showed that similarity coefficients among rep-PCR clusters within biovars are relatively low in many cases, indicating that disease outbreaks over time may have been caused by different clonal populations. The AFLP and sequence data (Cytocrome b561, Endoglucanase) confirmed these conclusions (Norman et al.,

2009). Similarly, all introductions of R3B2 strains into North America in the last few years have been linked to ornamental geranium production and appeared to be clonal.

We found evidence that new cool tolerant Biovar 1 strains of *R. solanacearum* entering the United States were genetically distinct from Biovar 1 strains currently identified infecting vegetable production. These introduced Biovar 1 strains targeted a broader host range and could infect not only tomato, tobacco, and potato but also cause symptoms on banana and the ornamental crops anthurium and pothos.

We identified additional populations beyond R3B2 strains of *Ralstonia* that are also capable of infecting plants under cool weather conditions (Bocsanczy et al., 2012). Our proteomics study revealed approximately 100 genes whose expression was temperature dependent and approx. 20 proteins with differential expression in cool virulent strains and non-virulent ones. The comparative protein analysis provided evidence that regulation at low temperature of the identified proteins provides a competitive advantage for inciting infection under cool weather conditions to cool virulent strains (Bocsanczy et al., submitted). This biological information on the adaptation of certain strains will be useful in the development of new control strategies.

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