Biology and Pathogenesis of Ralstonia solanacearum Race 3 on Geraniums

PRINCIPAL INVESTIGATOR:
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PROJECT OBJECTIVES:
The R3bv2 subgroup of the plant pathogen, *Ralstonia solanacearum,* is a quarantine pest and a listed Federal Select Agent. Accidental introductions of R3bv2 to the United States in infected geranium cuttings disrupted ornamental production and inflicted large losses. Our goals are to improve methods for detection and exclusion of this bacterium and to better understand the biology of its spread, infection, and persistence.

ACCOMPLISHMENTS:
- We showed that the commonly-used PCR assay for R3bv2 can give false positives; as a result APHIS instituted a more robust 2-step diagnostic protocol that combines PCR with a biovar test.
- We surveyed and characterized R3bv2 strains from the Guatemalan highlands and determined in field tests there that this pathogen is not usually transmitted through tomato fruit or seed; this had an indirect effect on floriculture producers.
- Our genome sequence of *R. solanacearum* R3b2 strain UW551 is driving new research into the biology of this pathogen and especially into badly needed new diagnostic methods. We are currently field-testing a set of highly R3bv2-specific diagnostic PCR primers in combination with LAMP, a rapid, robust detection method developed in collaboration with A. Alvarez.
- We tested the frequent assertion that R3bv2 survives cold longer than other *R. solanacearum* strains. Surprisingly, we found that native North American *R. solanacearum* strains survive longer at 4C than R3bv2, and that R3bv2 does not have a lower optimum growth temperature in culture. However at cool temperatures, R3bv2 is more virulent and survives longer in plants, indicating that its distinctive epidemiological traits are expressed in interaction with host plants and aren't associated with improved ennvironmental persistence.
- We are trying to identify the biological basis of the cool-temperate phenotype of R3bv2 strains using transcriptomics to compare gene expression between tropical and R3bv2 strains at 20° and 28°C. Testing of several putative cold-epidemiology genes is underway.
- We are characterizing survival of R3bv2 in geranium, tomato, and potato tissue at sub-zero and fluctuating temperatures; preliminary results indicate it cannot survive multiple freeze-thaw cycles even when protected by plant tissue.

TECHNOLOGY TRANSFER/IMPACT:
Our findings have helped shape the USDA-APHIS regulations and diagnostic standards for exclusion and detection of R3bv2 in geranium cuttings (Swanson et al 2007). Since 2006, I have consulted regularly with both CPHST scientists and representatives of the floriculture industry to improve offshore sanitation protocols and to resolve several false-positive reports of R3bv2 in

North America (e.g. Hong et al 2008). I have also been active in efforts by the scientific community to evaluate the severity of the security threat posed by R3bv2 (Young et al 2008) and to quantify the ability of R3bv2 to survive conditions in northern temperate winters (Milling et al 2009). Finally, we have worked to educate growers, regulators, and academics about the biology of this pathogen (Champoiseau et al 2009). In addition to the peer-reviewed publications listed below, I have also given invited talks at various national and regional industry and academic meetings.

Milling, A., F. Meng, T. P. Denny, and C. Allen. 2009. Interactions with hosts at cool temperatures, not cold tolerance, explain the unique epidemiology of *Ralstonia solanacearum* Race 3 biovar 2. Phytopathology 99:1127-1134.

Champoiseau, P., J. Jones, and C. Allen. 2009. *Ralstonia solanacearum* Race 3 biovar 2 causes tropical losses and temperate anxieties. Plant Health Progress, published 3/13/09.

J. M. Young, C. Allen, et al. 2008. Plant pathogenic bacteria as bioterror weapons: A real threat? Phytopathology 98:1060-1065.

Hong, J.C., T. Momol, J. Jones, P. Ji, S. Olson, C. Allen, A. Sanchez-Perez, P. Pradhanang, K. Guven. 2008. Detection of *Ralstonia solanacearum* in irrigation ponds and aquatic weeds associated with ponds in North Florida. Plant Disease: 92:1674-82.

Sanchez-Perez, A., L. Mejia, M. Fegan, and C. Allen. 2008. Diversity and distribution of *Ralstonia solanacearum* strains in Guatemala and rare occurrence of tomato fruit infection. Plant Pathology 57:320-331.

Swanson, J. K., L. Montes, L. Mejia and C. Allen. 2007. Detection of latent infections of *Ralstonia solanacearum* Race 3 biovar 2 in geraniums. Plant Disease 91:828-834.

ADDITIONAL FUNDING/EXTERNAL SUPPORT:

University of Wisconsin-Madison College of Agriculture support for graduate students working on this project, and generous donation of research materials from Ball FloraPlant, Sklarzyk Seeds LLC,and Agdia, Inc. In addition, I'm a collaborator on a separate CSREES integrated project on R3bv2 on potato and tomato. Although this FNRI project got no direct funding from the CSREES grant, it does benefit from intellectual synergy between the two projects. The floriculture industry and associated government regulators are beneficiaries of the education portion of this integrated project (see for example: http://plantpath.ifas.ufl.edu/rsol/index.html)

COLLABORATORS:

Timur Momol, David Norman, and Jeff Jones (University of Florida); Timothy Denny and Mark Schell (University of Georgia); Anne Alvarez (University of Hawaii); Luis Mejia and Amilcar Sanchez-Perez (U. San Carlos, Guatemala); Mike Klopmeyer (Ball FloraPlant); and Darryl Thomas (Goldsmith Seeds).