Understanding the “cool-temperate” epidemiology of *Ralstonia solanacearum* Race 3 biovar 2 on geraniums and other hosts

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Introduction

R3bv2 strains of the plant pathogen *Ralstonia solanacearum* cause potato brown rot disease, a major problem in the hothiipic tropics and in some temperate zones. These strains are described as more cool-tolerant than other *R. solanacearum* strains, which are warm-temperate/tropical. Establishment of R3bv2 strains in North America could threaten the U.S. potato industry. Thus the R3bv2 subgroup is a quarantine pest and a listed Federal Select Agent. This pathogen can also infect geraniums, which are commonly produced in regions where R3bv2 is endemic. Accidental introductions of R3bv2 to North America in infected 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.

Recent Accomplishments

1. We tested the frequent assertion that R3bv2 survives cold better than other *R. solanacearum* strains. Surprisingly, we found that:
   * Native U.S. *R. solanacearum* strains survive longer at 4°C than R3bv2 (Figure 1; Table 1).
   * At cool temperatures, R3bv2 survives longer in plants (Figure 2) and is more virulent (Figure 3).
   * Thus, its distinctive epidemiological traits do not derive from improved cold survival.

2. To identify the biological basis of the cool-temperate phenotype of R3bv2 strains, we compared gene expression between tropical and R3bv2 strains at 20°C and 28°C.
   * Microarray analysis identified some R3bv2 genes differentially expressed at 20°C versus 28°C (Table 2).
   * Mutant biological strains lacking some of these putative cold-epidemiology traits appear to be less virulent than wild-type at 20°C (Figure 4).

3. We are characterizing survival of R3bv2 in geranium, tomato, and potato tissue at sub-zero and fluctuating temperatures.
   * Preliminary results indicate that even when protected by plant tissue R3bv2 cannot survive the multiple freeze-thaw cycles typical of natural environments (Figure 5).

Other Accomplishments Since 2006

- 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 biotest.
- We showed that neither bacteriological and molecular tests can reliably detect R3bv2 in effluent water from latently infected geranium plants.
- We surveyed and characterized R3bv2 strains from the Guatemalan highlands and determined that this pathogen is not usually transmitted through tomato fruit or seed; this had an indirect effect on floriculture producers.
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