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Validation of confirmatory real-time PCR diagnostic assays for detecting *Phytophthora ramorum*

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One of the limitations of validated PCR assays currently used by PPQ to complete a diagnosis of *Phytophthora ramorum* (PR) is that each assay can cross-react with DNA from PR relatives if these are present at high titer, or if the validated process is not followed exactly. We have worked to validate a recently developed Real-time PCR assay diagnostic for PR for use by PPQ. This assay targets an intron from a single-copy *Phytophthora* gene (*Ypt1*). Sequence comparisons for *Ypt1* between PR, *P. foliorum* and *P. hibernalis* indicate that the target sequences for the primers and probe used in this assay differs by ~20% between PR and *P. foliorum*, and by ~30% between PR and *P. hibernalis*. The assay does not cross-react with DNAs of *P. foliorum* or *P. hibernalis* in controlled tests, or with environmental samples of these two species identified during 2006 surveys for PR. However, the *Ypt1* assay is less sensitive for PR DNA than the validated Nested or Real-time PCR assays that target the ITS regions. Due to the lower assay sensitivity and higher probability of false negative diagnoses, we do not recommend it as a stand-alone diagnostic for PR. The *Ypt1*-based assay is straightforward to conduct, does not cross-react with DNAs from *P. foliorum* and *P. hibernalis*, and does appear to be a useful confirmatory assay for PR.

A summary of National Survey and Compliance Testing for *Phytophthora ramorum* by NPGBL – 2005–2006

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Routine diagnoses of samples provided as part of operational testing for *Phytophthora ramorum* (PR), causal agent of Phytophthora blight and of Sudden Oak Death, during 2005 and 2006 have utilized a combination of validated conventional and Real-time PCR diagnostics. Over this period, we have used 3 PCR assays to test >3100 sample DNAs from 43 states, and from >55 plant genera. In both years the most commonly submitted samples were from *Rhododendron* (excluding Azalea), and were also the most often diagnosed as positive for PR. Other host genera frequently diagnosed as positive for PR included *Camellia, Kalmia, Pieris* and *Viburnum*. Samples from other hosts were rarely submitted for testing, or were rarely or never diagnosed as positive. PR positive samples were not evenly distributed across the USA. Greater than 90% of all PCR positives were received from sites in CA, OR or WA. Other positive diagnoses were rare, broadly distributed among states, and could be traced to known sources. Our data suggest that PPQ efforts since 2004 to restrict movement and establishment of PR have been generally effective, but that vigilance needs to be maintained in order to confirm that the quarantine strategies in place maintain effectiveness.

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Three dominant resistance genes (Rsv1, Rsv3, Rsv4) differentially confer resistance against *Soybean mosaic virus* (SMV) strains. SMV P3 is reported to be an elicitor for Rsv1 mediated extreme resistance (ER) to SMV strain N (G2). Here we show that Rsv3, effective against SMV strain G-7, allows limited movement of GUS-tagged SMV G-7 on Rsv3 genotype soybean. The resistance is neither ER nor hypersensitive. To identify virulence factors of Rsv3 mediated resistance, chimeras were constructed by exchanging fragments between SMV-G7 and SMV-N. Reciprocal replacements of the 5′ end 3.8kb fragment including genes P1, HC-Pro, P3 and the N terminus CI resulted in no infection. Additional constructs showed that the N-terminal region of CI but not P1, HC-Pro, and P3 is necessary, but not sufficient, for Rsv3 mediated resistance. Interestingly, tests showed this region is a symptom determinant. Severe symptoms were induced by SMV-N. Mild symptoms were induced when the homologue N terminus CI of SMV-N was replaced by that of SMV-G7. Three amino acid differences occur in this region between SMV-G7 and SMV-N. The first amino acid is a predicted CK2 kinase substrate. Point mutations showed that the predicted substrate amino acid is a symptom determinant.

Preliminary molecular characterization of two novel *Erwinia amylovora* type III secretion pathogenicity islands (T3SS-PAIs)

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Fire blight, caused by *E. amylovora*, is a particularly devastating bacterial disease of apples and pears. Early studies in elucidating the molecular basis for pathogenesis have identified an essential virulence system - the *hrp* type III secretion system (*hrp*-T3SS) in *E. amylovora* which delivers effector proteins into host plants. Subtractive hybridization and genome sequence have revealed two novel T3SS PAIs (*Erwinia*-pathogenicity *i*slands, EPI1 and EPI2). The two PAIs are closely related to each other, have a significantly lower %G+C content (38.4 and 43.4% mol G+C, respectively, compared to 53.5% mol G+C content in the whole genome), and phylogenetically related to the tsetse fly endosymbiont *Sodalis glossinidius* SSR-1 and to human pathogens *Salmonella* SPI-1 and *Yersinia* Ysa T3SS-PAIs. In order to study the function, regulation and substrate specificity of EPI1 and EPI2, a PCR-based novel gene deletion approach was employed to generate whole island deletion mutants. Pathogenicity assay with immature pear fruit and apple seedlings showed that EPI1 and EPI2 are not involved in virulence in plants. These results indicated that both EPI1 and EPI2 are acquired by *E. amylovora* through horizontal gene transfer and may function during interaction with insect vectors. Future studies are needed to elucidate the role of EPI1 and EPI2 during interaction with insect vectors.