Molecular Markers for Identification of *P. ramorum* and other *Phytophthora* spp. from Diseased Tissue

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The use of molecular methods for detection of plant pathogens can reduce the time required to process samples as well as improve the accuracy of pathogen identification. Rather than focus on identifying a single species, methods that focus first on identification at a genus level followed by a nested species-specific detection could facilitate identification of a broader number of pathogens. For example, while *P. ramorum* is the causal agent of SOD, it is not uncommon to recover *P. nemorosa* or *P. pseudosyringae* from forest samples exhibiting symptoms similar to those caused by *P. ramorum*. The diversity of *Phytophthora* spp. recovered from nursery samples causing similar symptoms would be even greater. Having a marker system that would first accurately determine if a *Phytophthora* spp. was present and then allow identification to a species level would simplify sample processing as well as provide additional data on the diversity of *Phytophthora* spp. present on the hosts under investigation.

A Species-specific PCR detection system was developed based on amplification of the spacer region between the mitochondrially-encoded *cox* I and II genes of *Phytophthora* using genus-specific primers developed from conserved regions of the flanking genes (Martin, F.N., Tooley, P.W. and Blomquist, C., 2004, Phytopathology 94: 621-631). A two-step multiplex amplification procedure is used for determining if a *Phytophthora* sp. is present in symptomatic plant tissue and clarifying if it is *P. ramorum*, *P. nemorosa* or *P. pseudosyringae*. The first round multiplex amplification contains two primer pairs, one for amplification of plant sequences to serve as an internal control to ensure that extracted DNA is of sufficient quality to allow for PCR amplification and a second primer pair specific for amplification of sequences from *Phytophthora* spp. The products of the first round amplification are diluted and amplified with primer pairs nested within the genus-specific amplicon that are specific for either *P. ramorum, P. nemorosa* or *P. pseudosyringae*. Using purified pathogen DNA the limit of detection for *P. ramorum* using this marker system was approximately 2.0 fg of total DNA. More recent work with this marker system has modified it for use with real-time PCR and identified species-specific primers for 5 other *Phytophthora* spp. To facilitate the identification of species other than *P. ramorum, P. nemorosa* or *P. pseudosyringae*, several restriction enzymes were identified that digest the genus-specific amplicon and RFLP analysis can be used to differentiate most species.

An RFLP technique was developed for identification of unknown isolates. PCR primers spanning the mitochondrially-encoded *cox* I and II genes have been developed that are capable of amplifying most of the gene cluster in the genus *Phytophthora* (Phytopathology 94: 983-991). Digestion of the amplicons with restriction enzymes generated species-specific RFLP banding profiles that were effective for isolate classification to a species level.

Additional details on these marker systems can be found at http://pwa.ars.usda.gov/salinas/cipru/frank/phyto.htm