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Specific hybridization real-time PCR probes for *Phytophthora ramorum* detection and diagnosis

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Summary

Sudden Oak Death, caused *by Phytophthora ramorum,* poses a serious threat to native American oaks, and is also present in Europe where it has been isolated from numerous European ornamental plant nurseries. Its proven aggressiveness against plants in the Fagaceae and Ericaceae and the damage it has caused in North America have lead to it being assigned quarantine status. The timely and accurate detection of P. *ramorum* is a critical aid in the study of the epidemiology and biology of this pathogen. As a regulated organism, the availability of a sensitive and reliable assay is essential when attempting to achieve early detection of the pathogen. In this work, new specific hybridization probes for a real-time PCR amplification method were found to be rapid, robust and labour-saving, and proved suitable for routine use in a molecular diagnostic laboratory.

1 Introduction

In recent years, diverse molecular techniques have been developed for detection and identification of plant pathogenic Oomycetes (COOKE et al. 2000). The polymerase chain reaction (PCR) has long been used to detect non-culturable pathogens, such as viruses (WETZEL et al. 1992) and phytoplasmas (GUNDERSEN and LEE 1996). Taxon-specific PCR has been used as a method of screening for microbes such as mycorrhizal fungi (GARDES and BRUNS 1993) as well as plant pathogens, including *Phytophthora* species (KONG et al. 2003). Because specific primers are used to discern small amounts of microbial nucleic acids from a much larger quantity of host plant DNA, or because samples at times include PCR inhibitors, nested-PCR may be required to detect low levels of infection (GONTHIER et al. 2003).

Methods based on DNA sequence information hold great promise in P. *ramorum* detection; however, if used for field diagnosis, they must clearly distinguish P. *ramorum* from several other *Phytophthora spp.* that may be encountered, including P. *nemorosa, P. pseudosyringae, P. cinnamomi, P. syringae, P. hevea, P. cactorum, P. citricola* and others (MARTIN et al. 2004). Thus, a high research priority has been the development of a PCR-based detection method specifically for P. *ramorum*.

Successful primer design for pathogen detection requires that the target region be (i) unique to the organism of interest and (ii) conserved across populations of the organism

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