

Molecular Detection of *Phytophthora ramorum* by Real-Time PCR Using Taqman, SYBR[®] Green and Molecular Beacons with three genes

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Sudden oak death, caused by *Phytophthora ramorum*, is a severe disease that can affect numerous species of trees and shrubs. This pathogen has been spread via nursery stock and quarantine measures are currently in place to prevent further spread. Molecular assays have been developed to rapidly detect and identify *P. ramorum* but one difficulty encountered with some of these assays is the inability to reliably distinguish between *P. ramorum* and closely related species.

In order to overcome cross reactions, β -tubulin and elicitin regions from a collection of *Phytophthora* species were sequenced and searched for polymorphisms. New assays were designed using existing internal transcribed spacer (ITS) sequences as well as new β -tubulin and elicitin sequences. Primers specific to *P. ramorum* were designed to amplify a 171 bp fragment of β -tubulin and these were used in a real-time PCR assay in conjunction with molecular beacons, Taqman probes and SYBR[®] Green in order to compare the three reporter systems. The best performing system was also used to compare the three DNA regions.. The real-time PCR assays differentiated *P. ramorum* from the 65 *Phytophthora* species tested, including *P. lateralis*. The assays were also used with DNA extracted from plants infected with *P. ramorum*. Overall, ITS and elicitin Taqman assays had the best combination of sensitivity and specificity.

With the availability of the *Phytophthora ramorum* genome, more genes were analysed for polymorphisms. Multiple intraspecific and interspecific polymorphisms were found in most genes assayed. Genotyping assays are currently being developed to allow simultaneously diagnosis at the species level as well as for multilocus fingerprinting of individuals. This should be a useful tool in understanding the origin and migration of *P. ramorum*.