

Application of Rapid Onsite PCR (TaqMan[®]) for *Phytophthora ramorum* Under U.S. Conditions¹

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Abstract

Currently, diagnosis of *Phytophthora ramorum* involves sending samples to a laboratory for traditional isolation and morphological characterisation, and/or PCR analysis. This can take as long as 2 weeks from sampling to final diagnosis. However, the Plant Health Group, Central Science Laboratory, has produced on-site DNA extraction and real-time PCR (TaqMan[®]) methods, which use field-stable reagents for diagnosis of symptomatic infections of *P. ramorum*, in under 2 hours (Tomlinson and others, 2005). In March 2004 this method was evaluated, with the help of the University of California, Berkeley and the USDA Forest Service, on leaf and stem samples from 21 plant species collected from four sites in the vicinity of San Francisco, California. DNA was extracted from each sample using a Bio-Nobile QuickPick[™] plant kit and added to a TaqMan[®] reaction mix developed for on-site use. This contained reagents for specific amplification of *P. ramorum* DNA and a universal plant gene (cytochrome oxidase (COX), used as an internal reaction control). Each sample was subjected to thermal cycling in a Cepheid SmartCycler[®] and the real-time data from these reactions interpreted. The majority of testing was performed at the University of California, Berkeley, for logistical reasons. However, on one day, samples were tested in the field at China Camp State Park using the complete on-site protocol in less than 2 hours, confirming this protocol can be performed independent of all laboratory support. At the University of California, Berkeley, plant material was plated out onto a *Phytophthora*-specific media (PARP) for comparative purposes to determine the presence of *P. ramorum* in each sample. COX amplification was achieved for all 21 host species with *P. ramorum* being detected by TaqMan[®] and isolation from leaf samples of tanoak, Douglas-fir, honeysuckle and bay laurel. *P. ramorum* was also identified by TaqMan[®] from stem samples of madrone, Shreve's oak, tanoak, redwood and Douglas-fir, but only isolated from madrone and tanoak. This study showed that real-time PCR diagnosis of *P. ramorum* can be performed on-site in under 2 hours on symptomatic stem and leaf material. This protocol will be further developed in the E.U. Portcheck project that aims to develop on-site methods for diagnosis of E.U. plant pathogens at national inspection points. Its use for on-site asymptomatic detection for *P. ramorum* is also underway.

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Reference

Tomlinson, J.A.; Boonham, N.; Hughes, K.J.D.; Griffin, R.L.; and Barker, I. 2005. **On-site DNA extraction and real-time PCR for detection of *Phytophthora ramorum* in the field.** Applied and Environmental Microbiology 71: 6702-6710.