Application of rapid on-site PCR (TaqMan®) for *Phytophthora ramorum* under US conditions

Kelvin Hughes, Jenny Tomlinson, Neil Boonham, Plant Heath Group, Central Science Laboratory (CSL), York, UK. YO41 1LZ, * k.hughes@csl.gov.uk, Tel. +44 1904 462000; Kelly Ivors and Matteo Garbelotto, Dept of ESPM-ES, University of California, Berkeley, Berkeley, California, USA & Ian Barker, Plant Heath Group, Central Science Laboratory (CSL), York, UK. YO41 1LZ

Currently diagnosis of *Phytophthora ramorum* involves sending samples to a laboratory for traditional isolation and morphological characterisation, and / or PCR analysis. This can take up to two weeks from sampling to final diagnosis. However CSL have 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. In March 2004 this method was evaluated, with the help of the University of Berkeley and the USDA Forest Service, on leaf and stem samples from 20 plant species collected from 5 sites around San Francisco, California, USA. 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 in 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 UC 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 20 host species with *P. ramorum* being detected by TaqMan® and isolation from leaf samples of tan oak, Douglas fir, honeysuckle and bay laurel. *P. ramorum* was also identified by TaqMan® from stem samples of madrone, Shreve's oak, tan oak, redwood and Douglas fir but only isolated from madrone and tan oak.

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 EU Portcheck project which aims to develop on-site methods for diagnosis of EU plant pathogens at national inspection points. Its use for on-site asymptomatic detection for *P. ramorum* is also underway.

Acknowledgements
Funding for this work was provided by Defra Plant Heath Division. The authors of this work wish to thank Cepheid, USA for the use of a loan SmartCycler® for this work.