

Comparative evaluation of real-time PCR (TaqMan[®]) with isolation for diagnosis of *Phytophthora ramorum*.

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Field samples (323 in total) were tested for *Phytophthora ramorum* by isolation and CSL's real-time PCR TaqMan[®] assay. The TaqMan[®] assay consists of reagents for specific amplification of *P. ramorum* DNA and a universal plant gene (cytochrome oxidase) as an internal reaction control. Samples were surface decontaminated and split into two equal parts. One was plated onto semi-selective agar (PARPH) and examined for *P. ramorum* after 6-days incubation, while DNA was extracted from the other and tested with the TaqMan[®] assay. Over 98 % of samples gave identical results for both isolation and PCR, with 25 of the 323 samples (8 %) testing positive for *P. ramorum*. Six samples (2%) did not agree however: three were isolation negative and PCR positive for *P. ramorum*, indicating that *P. ramorum* present was dead; two were isolation positive but PCR negative for *P. ramorum* and PCR positive for the internal control, thus showing no *P. ramorum* DNA was extracted from these samples, or it was below a PCR detectable level; and one sample was isolation positive and PCR negative for *P. ramorum* and the internal control, showing no amplifiable DNA was extracted from this sample. This trial demonstrated that isolation and TaqMan[®] PCR were equally reliable and robust for diagnosis of *P. ramorum* from the UK plant material tested.