Detection of *Phytophthora ramorum* using QuickPick™ Plant DNA kit for DNA purification in the field.

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ABSTRACT

Phytophthora ramorum (*P. ramorum*) is a severe plant pathogen currently described as causing oak mortality (sudden oak death) and other plant diseases in USA and Europe. Fast and accurate diagnostic tests are required in order to characterize the distribution of *P. ramorum* and prevent its introduction to pathogen-free areas.

A rapid and simple method for DNA purification was developed by the Central Science Laboratory (CSL, UK) for use at the point of sampling, using PickPen® technology and a modified QuickPick™ Plant DNA kit protocol, followed by a real-time PCR (TaqMan) assay for *P. ramorum* (1). To our knowledge, this is the first description of a method for DNA purification and molecular testing for a plant pathogen carried out entirely in the field.

INTRODUCTION

Since 1995, oaks and tanoaks have been dying in California due to a disease referred to as Sudden Oak Death. Recently, *P. ramorum* has been identified as the pathogen that causes this disease, as well as ramorum leaf blight and ramorum dieback on a range of other species. *P. ramorum* has also been found in several countries in Europe, mainly on nursery plants (most commonly *Rhododendron* and *Viburnum*).

Presently, the diagnosis of *P. ramorum* involves sending samples to a laboratory for traditional isolation and morphological characterization and/or PCR analysis. It can take up to two weeks from sampling to final diagnosis and cause a substantial delay in preventing the spread of pathogen within affected areas or into pathogen-free areas.

To speed up the diagnosis, an on-site method for DNA purification and molecular testing was developed. An obligatory feature for the testing is that the analysis can be carried out at the point of sampling in the field independently of any laboratory facilities (centrifuge, laminar hood, etc). The DNA purification was performed using magnetic particle technology with QuickPick™ Plant DNA kit followed by real-time PCR detection of *P. ramorum.*

MATERIALS & METHODS

The QuickPick™ Plant DNA kit was used to purify DNA from a wide range of plant species (leaf/stem samples) in the field, followed by detection of *P. ramorum* by real-time PCR (TaqMan). The QuickPick™ Plant DNA kit protocol was slightly modified to remove the need for sample homogenisation, vortexing and centrifugation.

Approximately 15-25 mg of leaf/stem tissue was cut into small pieces (2 mm^2) using a disposable scalpel blade and placed in a microtube containing 35 µl of Plant DNA Lysis Buffer. Plant DNA Proteinase K solution (5 µl) was added and the sample mixed by gently flicking the tube, then incubated at 65°C for 10 minutes in a heating block. After incubation, the lysate was transferred to a well in the first row of a 96-well standard microplate containing Plant DNA MagaZorb™ Magnetic Particles (5 µl) and Plant DNA Binding Buffer (60 µl) and mixed by gentle shaking for 2 minutes. A PickPen® 8-M was used to transfer the Magnetic Particles and bound DNA through two washing steps (150 µl Plant DNA Wash Buffer), and into 100 µl Plant DNA Elution Buffer. The elution was carried out by incubation at ambient temperature for 5 minutes with occasional gentle mixing using the PickPen® with magnets withdrawn. Finally, the Magnetic Particles were removed. Purified DNA eluates were tested by multiplex real-time PCR (TaqMan) on a portable Cepheid SmartCycler II using *P. ramorum*-specific primers and probe, and plant cytochrome oxidase (COX) primers and probe to detect DNA from the host plant.

RESULTS

The DNA purification method described has been used to test a wide range of plant species at *P. ramorum* outbreak sites in the UK and California. Amplifiable DNA (from pathogen and/or host) has been successfully purified from leaf/stem samples from a variety of species (Table 1).

Table 1. The alphabetical listing of plant species used for RT-PCR amplification after DNA purification with QuickPick™ Plant DNA kit.

Species	Common name
Acer macrophyllum	Big leaf maple
Aesculus californica	California buckeye
Arbutus menziesii	Madrone
Corylus sp.	Hazel
Heterolmeles arbutifolia	Toyon
Lithocarpus densiflorus	Tanoak
Lonicera hispidula	California honeysuckle
Magnolia sp.	Magnolia
Parrotia persica	Persian ironwood
Pinus sp.	Pine
Pseudotsuga menziesii	Douglas fir
Quercus agrifolia	Coast live oak
Quercus kelloggii	California black oak
Quercus parvula var. *shrevei*	Shreve's oak
Rhamnus californica	California coffeeberry
Rhododendron sp.	Rhododendron
Rubus spectabilis	Salmonberry
Sequoia sempervirens	Coast redwood
Syringa sp.	Lilac
Umbellularia californica	California bay laurel
Vaccinium ovatum	Evergreen huckleberry

As an example, genomic DNA was purified from both healthy and *P. ramorum* -infected *Rhododendron* sp. leaves using a PickPen® 8-M and QuickPick™ Plant DNA kit followed by real-time PCR pathogen detection. The whole procedure from DNA purification to diagnosis took less than 2 hours. The multiplex real-time PCR results clearly show the amplification of *P. ramorum* template in the infected rhododendron sample (Fig. 1). A universal plant gene, cytochrome oxidase (COX) was used as an internal reaction control in the analysis. The thermal cycling was performed with portable Cepheid SmartCycler® II.

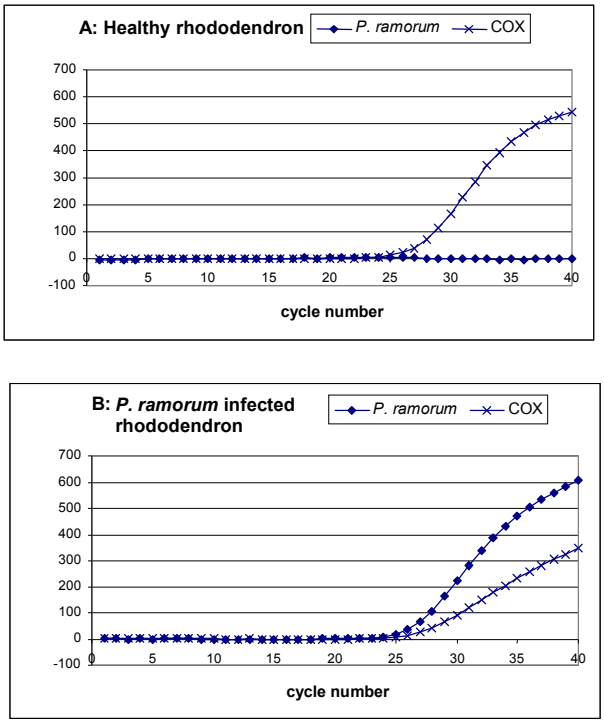

Figure 1. Multiplex real-time PCR analysis of healthy (A) and *P. ramorum* infected (B) rhododendron samples. A universal plant gene, cytochrome oxidase (COX) was used as an internal reaction control. The thermal cycling was performed with Cepheid SmartCycler® II.

CONCLUSION

An important requirement for working in the field is to overcome the lack of typical laboratory facilities. This was made possible by the use of magnetic particle technology with a PickPen® 8-M device and QuickPick™ Plant DNA kit, combined with a portable real-time PCR platform.

According to the results, the QuickPick™ Plant DNA kit generates high-quality DNA suitable for sensitive diagnostic analyses such as multiplex real-time PCR.

REFERENCE

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