A high impact role for the Freedom4 device in Forest Protection

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Introduction

In New Zealand there has been a long association of *Phytophthora* disease in forest trees (Scott & Williams, 2014) for example *Phytophthora cinnamomi* and *Phytophthora cactorum* in *Pinus radiata* forest nurseries and shelterbelts. However, the emergence of new arboreal *Phytophthora* diseases is increasing worldwide. In New Zealand new diseases of trees caused by *Phytophthora* species include red needle cast in *P. radiata* caused by *Phytophthora pluvialis* (Dick, et al., 2014; Reeser, et al., 2013) and kauri dieback in *Agathis australis* caused by *Phytophthora agathadicida* (Weir, et al., 2015).

Kauri (*Agathis australis*) are a native taonga (treasure) tree species in New Zealand and hold cultural significance for all New Zealanders being associated with the history and legends of Māori. The largest Kauri are referred to by name (e.g. Tāne Mahuta, Lord of the Forest) and revered as chiefs.

Considerable expertise is required to diagnose an infected tree by its symptoms, especially if multiple diseases are present in one forest. Also, trees can be located in quite remote locations. Standard diagnostic tests require infected material to be transported to a central laboratory, risking disease spread, and the tests can take weeks to perform. Quantitative real-time PCR (qPCR) assays have been developed to detect *P. agathidicida* (Than, et al., 2013) and *P. pluvialis* (McDougal et al., unpublished) in response to this challenge. If qPCR technology could be taken to trees, suspected of being infected, diagnosis would be faster. Overall, this would result in *Phytophthora* outbreaks being more rapidly contained.

Disease management for infected forests is difficult. One option for effective control is the development of resistant cultivars. Mobile qPCR would facilitate identification of wild trees that are resistant to disease despite exposure to the pathogens. It would also enable accurate diagnosis of disease required for assessment of *P. radiata* field-trials testing selected genotypes for resistance. Drs Rebecca McDougal and Emily Telfer from Scion Ltd, New Zealand are interested in technologies that bring reliable molecular testing for *Phytophthora* to the tree. The Freedom4 is a mobile qPCR instrument ideally suited to this task. Here we show that the Freedom4 is able to perform qPCR assays for *P. agathidicida* and *P. pluvialis* detection.

Materials and Methods

qPCR instruments

Reactions were performed in parallel on the Freedom4 (Ubiquitome, New Zealand) and the LightCycler 480 (Roche, Switzerland).

Template

Six DNA templates were supplied by Drs McDougal and Telfer, three for use with each pathogen assay. Sample DNA was extracted from two different materials, P. radiata needles and kauri dieback soils. Details are given in Table 1.

qPCR amplification chemistry and assays

All work reported here used the LightCycler 480 2x SYBR Green Master Mix I system from Roche (Roche Cat No. 04 707 516 001). Probes and primers are given in Than et al (2013) and McDougal et al. (manuscript under review). All oligonucleotide probes and primers were synthesised by Integrated DNA Technologies (USA).

Reaction mixes consisted of 10µl 2x SYBR Green Master Mix I, 1µl each of Forward and Reverse primer (or 2µl of PrimeTime IDT qPCR assay mix), 1µl DNA template and PCR grade water

to a final volume of 20µl. The thermal cycle program used for all experiments was 95°C for five minutes to activate, followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds.

Results and Discussion

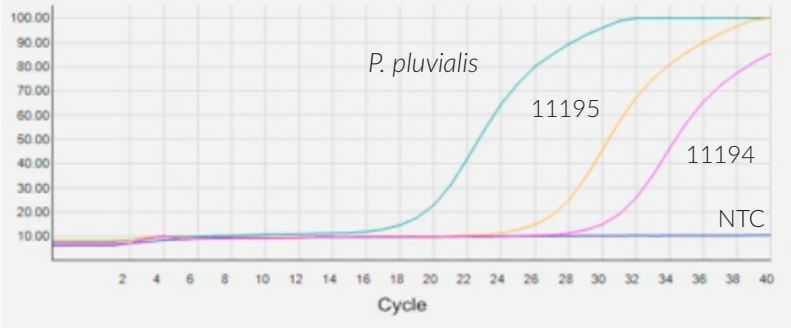
Figure 1 shows both the amplification curves and the Cq calls for the *P. pluvialis* qPCR assay used with *P. pluvialis* positive control DNA (NZFS3000) and the two DNA samples extracted from *P. radiata* needles, both known to be infected. qPCR was run on both the Freedom4 and the LightCycler 480 in parallel and both instruments gave comparable results. The positive control DNA was clearly positive and the DNA preparations from infected samples were also shown to contain *P. pluvialis*.

The *P. agathidicida* assay, in our hands, proved problematic giving the same results for both the LightCycler 480 and the Freedom4. The assay was performed with and without probe. No Cq calls were obtained from either instrument when probe was present. However, once the probe was omitted amplification and Cq calls were determined for all three templates. Cq calls and amplification curves are given in Figure 2.

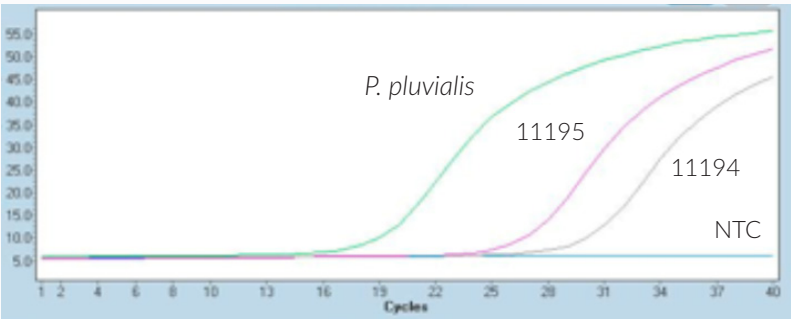
Table 1 Template details

Pathogen	DNA Culture (positive control)	DNA purified from contaminated/ infected material	DNA Extraction Method
Phytophthora pluvialis	NZFS 3000	Needle samples 11195 & 11194	Nucleospin Plant II system (Machery-Nagel GmbH, Düren, Germany).
Phytophthora agathadicidia	NZFS 3770	Soil sample extracts: J & K	Than et al., 2013

Freedom4



LightCycler 480



Template	Freedom4 Cq	LightCycler 480 Cq
P. pluvialis	19.90	18.97
11194	31.09	29.93
11195	27.50	26.67

Figure 1: *P. pluvialis* qPCR assay performed on the Freedom4 and the LightCycler 480. The *P. pluvialis* positive control was extracted from a pure *P. pluvialis* culture. 11194 and 11195 consisted of DNA extracted from infected *P. radiata* needles.

Examination of Figure 2 shows that there was higher background fluorescence for the soil-extracted samples J and K compared to the pure culture-derived *P. agathidicida* DNA positive control. This was evident on both the Freedom4 and the LightCycler 480. Sample DNA was extracted from soil using the method described in Than et al. (2013). In their publication, Than et al. (2013) found DNA extracted from soil exhibited an order of magnitude reduction in qPCR sensitivity. This would suggest that using the Than et al. (2013) method allows other soil-based contaminants to be retained in the sample. This may account for high background fluorescence in Sample J and K but not the positive control DNA. Interestingly, a difference in background signal between the positive control and the samples was not seen when DNA was extracted from pine needles. DNA from the *P. radiata* needle samples were extracted using a modification of the commercially available kit Nucleospin Plant II system (Machery-Nagel GmbH, Düren, Germany) (Telfer et al. 2013).

In conclusion, these results show that the Freedom4 is able to perform qPCR assays for *P. pluvialis* and *P. agathidicida* detection as well as the LightCycler 480.

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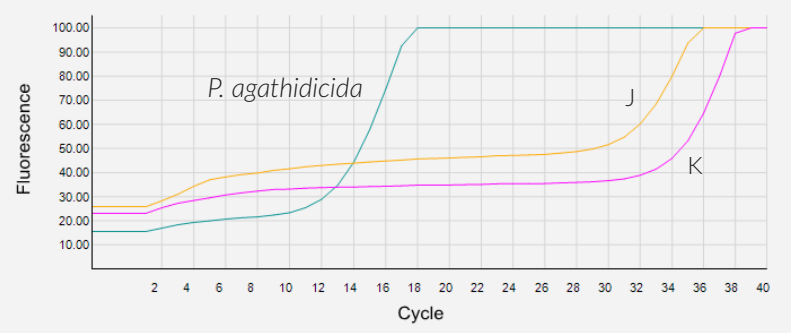
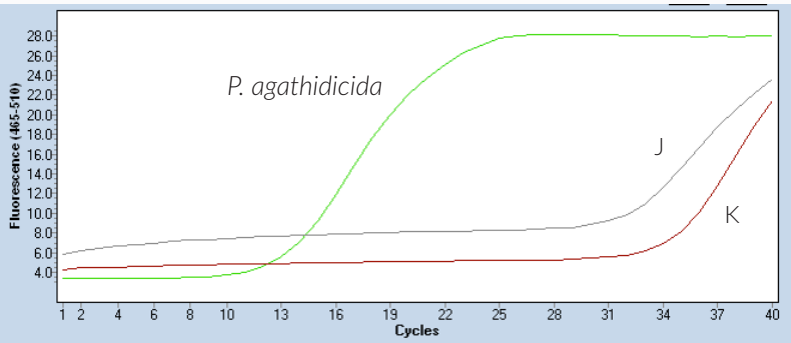
Freedom4

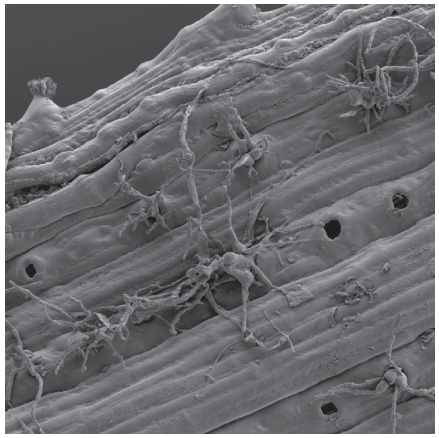


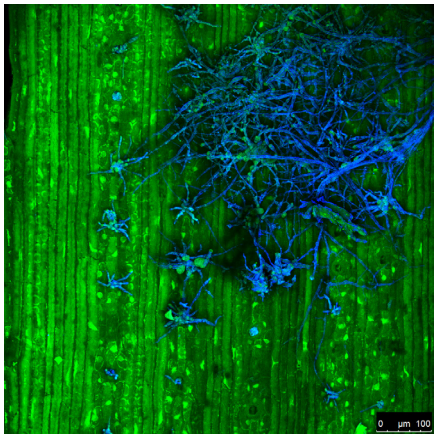
LightCycler 480



Template	Freedom4 Cq	LightCycler 480 Cq
P. agathidicida	13.44	13.09
Sample J	31.95	32.02
Sample K	34.56	+35

Figure 2: *P. agathidicida* assay performed on the Freedom4 and the LightCycler 480. The *P. agathidicida* positive control was extracted from a pure *P. agathidicida* culture. Samples J and K consisted of DNA extracted from soil around an infected tree.





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