# COMPARISON OF BAITING AND MOLECULAR METHODS FOR THE DETECTION OF PHYTOPHTHORA CINNAMOMI FROM SOIL SAMPLES FROM SOUTH WESTERN AUSTRALIA

N. M. Anderson, G. Hardy and P.A. O'Brien
Biological Science and Biotechnology, Murdoch University, South Street, Perth 6150

## INTRODUCTION

*Phytophthora cinnamomi* has been responsible for widespread destruction of native forests and heath lands throughout southern Australia. Baiting has been the primary method for detecting *P. cinnamomi* from soil samples (1). However, it is limited by the common occurrence of false negatives and the inability to detect dormant spores within the soil (2). The introduction of molecular based detection methods offer improved sensitivity and specificity for *P. cinnamomi* detection (3). PCR amplification is central to various methods of molecular detection including PCR-RFLP and microarray analysis. Several previous studies have compared the theoretical sensitivity of PCR and baiting of zoospore and chlamydospore inoculations without assessing naturally infested soils (4). This study compares baiting to primary and nested PCR detection of *P. cinnamomi* from naturally infested soils.

## MATERIALS AND METHODS

Nested PCR primers were designed to target the ITS1 and 2 regions of *P. cinnamomi*. PCR detection was optimised to improve specificity and overcome the influence of inhibitory compounds from a range of soils sourced from throughout south Western Australia.

Efficiency of baiting and molecular detection trials were compared for naturally infested soils to determine the minimum amount of soil required to detect *P. cinnamomi*. The impact on detection of periods of warm and dry conditions was assessed by storing the soil for extended periods of time at 25 and 30°C whereby the detection analysis was repeated.

## RESULTS

PCR based analysis was found to be far more sensitive in detecting *P. cinnamomi* than baiting in each of the soils analysed. In many cases, PCR analysis enabled the detection of the pathogen when baiting failed to do so. PCR detection of soil baits also proved to be more sensitive than culturing of bait onto selective media. This was attributed to the baited *P. cinnamomi* being overgrown by non-target species when grown on antibiotic infused agar. However, PCR detection directly from the soil extracts was always more sensitive than PCR detection by baiting.

In cases where baiting was successful equivalent levels of detection could be achieved by PCR analysis using 0.5g of soil compared to 50g used for the baiting assays. The increased sensitivity of PCR detection enabled *P. cinnamomi* to be detected from 1g of after samples were stored at 25°C and 30°C for up to 12 weeks. No *P. cinnamomi* was recovered from baiting 64g of the same soil.

## DISCUSSION

Improved sensitivity and reliability of pathogen detection is central to the implementation of effective pathogen management. This study has shown that DNA based detection is more reliable for the detection of *P. cinnamomi* from soil samples. Furthermore the improved sensitivity of detection following periods of unfavorable conditions has been demonstrated. The significance and application of these results to the management of *P. cinnamomi* will be discussed.

## ACKNOWLEDGEMENTS

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