TRANSFORMATION OF *PHYTOPHTHORA CINNAMOMI* WITH THE GREEN FLUORESCENT PROTEIN (GFP) FOR STUDIES ON SURVIVAL

K.L. McCarren[1], J.A McComb[1], B. Shearer[2], P. O'Brien[1] and G.E.St.J. Hardy[1]
[1] School of Biological Sciences and Biotechnology, Murdoch University, Murdoch, WA 6150, Australia.
[2] Department of Conservation and Land Management, Perth, WA 6152, Australia.

INTRODUCTION

For soilborne plant pathogens such as *Phytophthora cinnamomi*, differentiating between spores of *P. cinnamomi* and other similar microorganisms can be challenging, making it difficult to determine the form the pathogen survives in soils and host tissue. Previous research has shown that the pathogen may be capable of producing thick-walled chlamydospores (1). However, due to the difficulty in germinating these structures routinely, presumably due to dormancy, research has not been able to progress in this area as it is unclear whether these are actually propagules of *P. cinnamomi*.

The transformation of *P. cinnamomi* with the Green Fluorescent Protein (GFP) provides the opportunity to study the growth and survival in plants and soil under non-sterile conditions.

The present study aimed to transform *P. cinnamomi* using the protoplast method to incorporate the GFP and geneticin resistance genes. The stability and pathogenicity of transformants were then compared with non-transformed isolates before studying formation and survival of chlamydospores under sterile and non-sterile conditions.

MATERIALS AND METHODS

Transformation of *P. cinnamomi*. The method by (2) was followed. Transformed isolates were maintained on V8 agar amended with geneticin. Colonies were checked for fluorescence.

Testing the effect of transformation on the growth of *P. cinnamomi*. Colony diameter of transformed and non-transformed cultures on 90mm Petri dishes with V8J agar or V8J agar containing 20μgml^{-1} geneticin was measured. Pathogenicity of transformants was determined by a lupin bioassay where 1-week-old lupins were inoculated with transformed and non-transformed cultures of *P. cinnamomi*. Lesions were measured.

Survival propagule studies. Roots of 1-week-old aseptically grown lupins were inoculated with transformed and non-transformed cultures of *P. cinnamomi*. After 2 weeks, colonised roots were then placed in 50mm Petri dishes containing 10ml soil extract. Plates were incubated for 3 weeks at 25°C. Roots were divided in half longitudinally and chlamydospores observed under both light and fluorescent (blue excitation at 480nm) microscopy.

RESULTS

P. cinnamomi was successfully transformed and two isolates were chosen for further detailed studies. The transformed isolates grew significantly ($p<0.001$) slower than their non-transformed parents. On V8J agar containing Geneticin, transformed isolates grew to a maximum diameter of 70mm whereas their non-transformed parents only grew to a maximum of 10mm diameter.

Pathogenicity testing of the transformed isolates showed a significant ($p<0.05$) reduction in root length colonised compared to their parents by 14% and 19% for MP103 and MP127, respectively.

Within lupin roots in a non-sterile environment, chlamydospores of *P. cinnamomi* fluoresced under blue excitation allowing them to be distinguished from spores of other fungi (Figure 1).

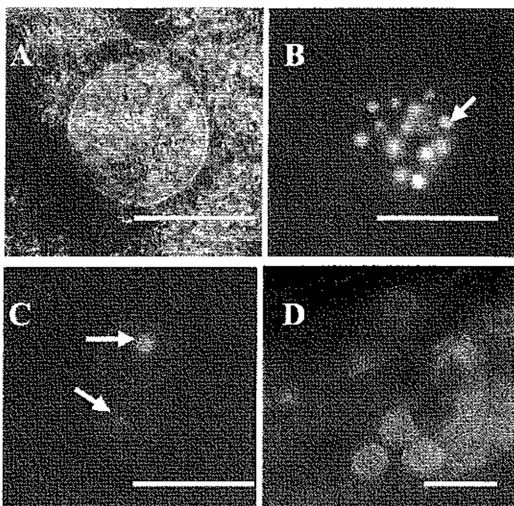

Figure 1: Chlamydospores within lupin roots in soil extract. **A** Chlamydospore under white light, **B** Same as A but fluorescing under blue light, **C** Fluorescing chlamydospores under blue light, **D** Non-fluorescent spores under blue light. Bar = 50 µm. Arrows indicate fluorescent material.

DISCUSSION

P. cinnamomi transformed with green fluorescent protein allowed chlamydospores to be easily distinguished from spores of other microorganisms in a non-sterile environment. Transformants, resistant to Geneticin, grew less readily on agar without antibiotic and were less pathogenic than their non-transformed 'parents' which supports studies by (3).

Future studies will determine the saprophytic ability of *P. cinnamomi* and whether thick wall chlamydospores do occur and their role.

ACKNOWLEDGEMENTS

We would like to thank F. Mauch (University of Fribourg, Switzerland) for providing the GFN plasmid. Also to Alcoa, Worsley Alumina and Murdoch University for their funding and support.

REFERENCES

1. Cother E.J., Griffin D.M. (1973) Formation of chlamdospores by *Phytophthora drechsleri*. *Trans. Brit. Mycol. Soc.* **61**: 379 - 402.
2. Judelson, H. S. *et al.* (1991). Transformation of the oomycete pathogen *Phytophthora infestans*. *Mol. Plant-Microbe Int.* 4(6): 602 - 607.
3. Si-Ammour, A. *et a*l. (2003). Quantification of induced resistance against *Phytophthora* species expressing GFP as a vital marker: β-aminobutyric acid but not BTH protects potato and *Arabidopsis* from infection. *Mol. Plant Path.* **4**(4): 237 - 248.