Role of the feral pig (*Sus scrofa*) in the dissemination of *Phytophthora cinnamomi* in south-western Australia

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Submitted by

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DECLARATION

The work described in this thesis was undertaken while I was an enrolled student for the degree of Doctor of Philosophy at Murdoch University, Western Australia. I declare that this thesis is my own account of my research and contains, as its main content, work which has not previously been submitted for a degree at any tertiary education institution.

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ABSTRACT

Phytophthora cinnamomi is a soil-borne plant pathogen that causes dieback, a disease that devastates many native vegetation ecosystems in Australia, particularly in south-west Western Australia. Feral pigs have long been implicated as vectors in the spread of this introduced plant pathogen due to their contact with infested soil and foraging habits. This study aimed to investigate the potential for feral pigs to disseminate *P. cinnamomi* and to determine their role in the spread of dieback.

Feral pigs trapped in three sampling areas within the northern jarrah (*Eucalyptus marginata*) forest of south-west Western Australia were sampled for the presence of *P. cinnamomi*. Faecal (n=208) and soil samples (n= 140) were collected from trapped pigs. In addition, 374 faecal and 36 soil samples were also collected from sites frequented by feral pigs. *Phytophthora cinnamomi* was not recovered from any of the faecal or soil samples. However saprophytic pathogens such as *Mucor* and *Fusarium* spp. were detected in the faeces and *Pythium* spp. was also detected in the soil samples, suggesting that feral pigs can act as vectors for the spread of soil-borne pathogens.

Stomach contents from 100 feral pigs trapped across the three sampling areas were analysed to investigate the proportion of *P. cinnamomi* susceptible plant matter present in the feral pig diet. A high frequency of plant material (85%) was found in the pig stomachs, of which 25.8% consisted of subterranean plant structures such as roots and tubers. Underground fruiting bodies of ectomycorrhizal fungi belonging to the genus *Rhizopogon* were also a significant food item. There was no statistically significant preference detected for food items by pigs between the three sampling areas, regardless

of sex and/or month of capture. However, older and larger pigs consumed significantly more bark (p= 0.0002).

To further investigate the potential for *P. cinnamomi* to survive passage through the pig digestive tract a feeding trial was undertaken. *Phytophthora cinnamomi* inoculated millet (*Panicum miliaceum*) seeds, pine (*Pinus radiata*) plugs, and *Banksia leptophylla* roots were fed to pigs and subsequently recovered after passage. The viability of *P. cinnamomi* inoculated plant materials post digestion ranged from 25.5% to 98.3%. Detection for *P. cinnamomi* presence in the materials via qPCR confirmed a decrease in *P. cinnamomi* DNA with increasing time to passage. These investigations demonstrated that plant material infected with *P. cinnamomi* can remain viable following passage through the pig digestive tract suggesting that the plant material may provide protection for *P. cinnamomi* against the adverse conditions of the pig digestive tract. Subsequently, plant infection trials using infected pine plugs passaged through the pig digestive tract highlighted that material passaged 7 days after initial consumption was capable of infecting healthy susceptible plants. This provides evidence that feral pigs have the ability to act as a vector for *P. cinnamomi* through the ingestion of infected plant materials.

A species-specific fluorescent *in situ* hybridization (FISH) assay was developed to enable the examination of *P. cinnamomi* within plant tissues. The probe was found to be specific for *P. cinnamomi* when tested against other *Phytophthora*, *Pythium* and enteric bacteria species. Using the FISH assay, the location of *P. cinnamomi* structures were detected within a variety of plant materials such as millet seeds, pine sections and root samples. *Phytophthora cinnamomi* structures such as hyphae and chlamydospores were found in the epidermal layer of millet seeds and within the axial rays of pine that were recovered after passage from the feeding trial. This aided understanding of how

viable *P. cinnamomi* were able to survive passage within these plant materials. In addition, the FISH assay was also successfully applied to both laboratory-cultured and naturally infected plant roots enabling detection of the pathogen in the intracellular and intercellular spaces of roots. The assay has proven to be a useful tool in the detection of *P. cinnamomi* structures within plant tissues.

In conclusion, this study provides evidence that, whilst the potential consequences of pig-vectored dispersal of *P. cinnamomi* are high, the likelihood of feral pigs dispersing the pathogen through transport of infested soil is low. Investigations of their diet composition and the passage of viable *P. cinnamomi* has established the additional threat that feral pigs could spread ingested *P. cinnamomi* within organic substrates. This study has also highlighted the fact that there is still much to be learned about the interaction between the feral pig and the plant pathogen. Further research is therefore required to ensure that appropriate management decisions for both species can be made.

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"Through a study in da…best, dirty south-WEST…

PhD = ***Ph****ytophthora* **D**ieback; **P**ig **h**og **D**ung; **P**ig **h**arbour **D**ieback"

Thesis is dedicated to all ma fallen soldiers…Rise up, persevere and battle on…

"Do not be pushed by your problems; be led by your dreams...

Dear Tomorrow, do whatever you want to do…

I have already lived my Today and…

I am not afraid of you…

Anymore…"

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