

**Occurrence and variation of
Endothiella eucalypti in
Eucalyptus globulus plantations of
south-western Australia and the
influence of some biotic and abiotic
factors on the response of the host to
the pathogen.**



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Declaration

I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary education institution.

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Abstract

As the *Eucalyptus globulus* plantation industry expands and matures in southwestern Australia (WA), the impact of disease within the plantation environment is predicted to increase. This thesis investigated the most abundant canker-causing pathogen associated with branch and stem cankers, *Endothiella eucalypti* the anamorph of *Cryphonectria eucalypti*. *Endothiella eucalypti* was widespread, although at low incidence, throughout the WA plantation estate and was frequently observed sporulating on the bark of healthy hosts in the absence of disease. Regions with a long (approximately 20 years) plantation history, such as Bunbury, had the highest incidence of this pathogen. A high degree of variability in pathogenicity, growth rate and colony morphology was observed between WA isolates of *En. eucalypti*.

In the glasshouse, a significant variation in susceptibility of seven *E. globulus* provenances to *En. eucalypti* was observed. Although an interaction between the *E. globulus* provenance and *En. eucalypti* isolate was recorded, some provenances were generally more susceptible than others. In two 18-month-old plantations, the susceptibility of three provenances to *En. eucalypti* was significantly influenced by environmental conditions. Visual assessment of general tree health indicated that less healthy trees had smaller lesions than healthy trees. It is hypothesised that the selection of *E. globulus* provenances to suit site conditions in the future should decrease the risk of serious disease, especially on marginal sites.

Endothiella eucalypti caused disease in intact stems of two-year-old *E. globulus* under glasshouse conditions. This suggests that *En. eucalypti* may not require a wound to infect in the field.

Vegetative compatibility groupings between WA *En. eucalypti* isolates indicated a relatively high degree of genotypic diversity within the WA asexual population of *En. eucalypti*, whereas inter-simple sequence repeats PCR (ISSR-PCR) analysis indicated a lower level of genotypic diversity. Discrepancies between traditional and molecular techniques, such as ISSR-PCR, was attributed to the more specific gene-to-gene analysis afforded by molecular techniques. ISSR-PCR successfully distinguished variability within the *En. eucalypti* population and with the teleomorph, isolated in South Africa. It also separated *Cryphonectria cubensis* isolates from the *C. eucalypti* isolates.

As copper is the micronutrient most limiting growth of *E. globulus* in WA, its role in the resistance of two *E. globulus* provenances was examined in a glasshouse trial. Lesion extension or defence responses of *E. globulus* to *En. eucalypti* did not differ between Cu-adequate and Cu-deficient plants. It is suggested that constitutive levels of host defence enzymes played a more important role in providing protection for the host against *En. eucalypti* than the external supply of copper.

A reduction in the canopy volume of *E. globulus* within plantations due to insect herbivory or foliar pathogens, such as *Mycosphaerella* spp., has been reported to predispose the host to disease caused by non-aggressive canker-causing fungi. Under two separate glasshouse trials, conditions of 100% defoliation and 80% defoliation maintained over six weeks prior to inoculation, were required to significantly increase lesion extension caused by *En. eucalypti* in *E. globulus* stems. The ability of defoliated *E. globulus* to retain a degree of resistance to *En. eucalypti* was attributed to the rapid replacement of foliage and up-regulation of photosynthesis in remaining leaves. The carbohydrate reserves of the plant were depleted following defoliation and remained depressed regardless of the length of time the trees remained defoliated.

In conclusion, the endophytic habit of *En. eucalypti* poses a threat to highly stressed trees, however it does not appear to be an immediate threat to WA plantation health. Although *En. eucalypti* has not yet been responsible for a major disease outbreak in WA, the impact of this disease on plantation-grown eucalypts elsewhere in Australia and worldwide serves as an indication of its potential to affect WA plantations.

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Abbreviations

ANOVA	analysis of variance
BSA	bovine serum albumin
CHV	Cryphonectria hypovirus
CI	consistency index
CRY	<i>Cryphonectria cubensis</i>
Cu ⁺	fertiliser treatment with copper
Cu ⁻	fertiliser treatment without copper
DCLM	Department of Conservation and Land Management
DDI	double de-ionised
DNA	deoxyribonucleic acid
dsDNA	double-stranded deoxyribonucleic acid
dsRNA	double-stranded ribonucleic acid
EDTA	ethylenediamine tetracetic acid
FW	fresh weight
G	genotypic diversity
IBDU	isobutylidene diurea
ICPAES	inductively-coupled plasma atomic emission spectrometry
ISSR	inter-simple sequence repeats
ITC	Integrated Tree Cropping Ltd
LSD	Least significant difference
MLD	<i>Mycosphaerella</i> leaf disease
NA	not available
NB	no bands
P	phosphate
PCR	polymerase chain reaction
PDA	potato dextrose agar
PDA+S	potato dextrose agar containing streptomycin sulfate
PO	peroxidase
PVC	polyvinylchloride
PYC	pycnidia
RAMS	randomly amplified microsatellites
RFLP	restriction fragment length polymorphism
RI	retention index
RNA	ribonucleic acid
RNase	ribonuclease
SDS	sodium dodecyl sulfate
SE	standard error
SOD	superoxide dismutase
SSR	single sequence repeats
T	transect
VC	vegetative compatibility
WA	Western Australia
YFEL	youngest fully expanded leaf
YML	young mature leaves