The potential role of a *Phytophthora* species in *Eucalyptus gomphocephala* (tuart) decline in southwest Western Australia

Peter Scott,[1,2,6] Paul Barber,[1,2] Treena Burgess,[1,2] Bryan Shearer,[3] Ian Colquhoun,[4] Thomas Jung,[5] and Giles Hardy[1,2]

[1] Centre of Phytophthora Science and Management, Murdoch University, Australia
[2] Tuart Health Research Group (www.tuarthealth.murdoch.edu.au), Murdoch University, Australia
[3] Science Division, Department of Environment and Conservation, Australia
[4] Alcoa Alumina Australia
[5] Thomastrasse 75 83098 Brannenburg Germany, (www.tree-diseases.com)
[6] Contact should be made to P. Scott, email P.Scott@murdoch.edu.au

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Introduction

Eucalyptus gomphocephala is a unique keystone canopy species endemic to a narrow (5-10 km wide) coastal strip approximately 300 km in length in the southwest of Western Australia. *E. gomphocephala* is undergoing a significant decline throughout large sections of its remnant distribution within Yalgorup National Park, and in some areas resulting in 100% mortality (Barber et al. 2007). Fine feeder root damage and root necrosis has been observed in declining *E. gomphocephala* across a range of sites. In addition, tree injection trials with phosphite, a chemical known to induce host defence responses to *Phytophthora* species, have resulted in increased crown vigour and health. These observations indicate that a *Phytophthora* species could be associated with tuart decline. The aim of the current study was to determine whether *Phytophthora* species are associated with *E. gomphocephala* decline.

Materials and Methods

The independent variables were the harvested trees and their associated root systems and rhizosphere soil at 16 sites throughout the dominant *E. gomphocephala* decline within Yalgorup National Park. The dependant variables were the isolation success and frequency of *Phytophthora* species from soil baiting and the species identification using molecular and morphological techniques. Soil sampling, baiting and isolation techniques were modified from Jung et al. (2000). Rhizosphere soils were baited in 35 x 35 cm trays pre-moistened for 12 hrs before flooding to three to four cm in depth and baiting with juvenile leaves of *Quercus ilex* and *Q. suber*. Water soaked lesions, appearing after 48 hrs, were plated onto PARPHN selective media modified to: pimaricin 10 mg L^{-1}, ampicillin 200 mg L^{-1}, rifampicin 10 mg L^{-1}, pentachloronitrobenzene 100 mg L^{-1}, hymexazol 25 mg L^{-1}, Nystatin 50 mg L^{-1}, 10% V8 broth and Agar 16 g L^{-1}. ITS sequences of genomic rDNA were extracted using primers ITS4 and DC6 (Cooke et al. 2000) and compared to alignments of *Phytophthora* species isolated from the southwest of Western Australia at the Centre of Phytophthora Science and Management. Oogonia, oospore and antheridia measurements were conducted as indicated in Jung et al. (1999) on 50 oogonia, oospores and antheridia of one isolate. Sporangia measurements were conducted on 30 sporangia at 400x magnification on 10-day-old cultures grown on 10% V8 agar, flooded just over the surface for 24 hrs with a non-sterile soil extract. Preliminary assessment of morphological characteristics of oogonia, oospores, antheridia and sporangia were compared to the standard mycological key, Stamps et al. (1990) and the original *P. citricola* description (Sawada 1927). Preliminary culture morphology and growth rate measurements were conducted on V8 agar at 17°C and 20°C.

Results

The *Phytophthora* isolates from declining *E. gomphocephala* sites all appear to be the same species using molecular and morphological techniques, and were isolated from two declining sites 3.38 km apart within a region of extensive decline inside Yalgorup National Park. Initial sequence alignments place the *Phytophthora* isolates closest to *P. citricola*. Similar isolates have also been isolated from across the southwest of Western Australia by the Vegetation Health Service at the Department of Environment and Conservation, Kensington Western Australia. Oogonia formed readily in single culture after four days, were borne terminally, had smooth walls and varied in shape from spherical to slightly ovoid. Oospores were spherical and nearly plerotic. Antheridia were obovoid or irregular, strictly paragynous, diclinous and typically formed attachments close to the oogonia stalk. Antheridia were rarely borne intercalary. The most common sporangia shapes were ovate or limoniform, typically non-papillate or occasionally semi-papillate, non-caducous and formed occasionally conspicuous basal plugs protruding into sporangia. Sporangia were borne with a terminal attachment on and occasionally formed a lateral attachment and formed from internal proliferation. Sporangia rarely formed multiple papillae or distorted shapes on V8 agar. Uniform culture morphology was observed on V8 agar up to 22 days at 20°C in the dark. Greater radial colony expansion was observed on V8 agar at 17°C compared to 20°C on PARPHN agar. This contrasts to the optimal growth rate of the *P. citricola* ex-holotype culture which is recorded as producing lanceolate shaped sector colony morphology with an optimal growth rate between 25 - 28°C. Reproductive measurements correspond to the holotype measurements (Sawada 1927) as indicated (Table 1).

Discussion

This work represents a first record of *Phytophthora* species associated with declining *E. gomphocephala*. Although the specific phylogeny and epidemiology of these isolates is presently ongoing, these findings highlight the possibility that a *Phytophthora* species may be contributing to the decline of *E. gomphocephala*. The observation that the isolates have a temperature optimum of 17°C not 25 - 28°C, as recorded for the *P. citricola* holotype, raises some interesting questions with regards to its epidemiology. It is possible that this new *Phytophthora* species is active during the winter months when conditions are wet and cold. The average maximum and minimum winter temperature for Mandurah Park, approximately 22 km from the isolation sites, ranges from 20.6-10.8°C in May, to 19.2-9.6°C in September. Significant work is now required to accurately clarify the phylogeny and role of this organism in the *E. gomphocephala* decline. This is particularly important considering the range of other factors already identified as potential contributors to the decline (Barber et al. 2007). These include changes to fire regimes, hydrology, climate change, insect pests, nutrition and fungal foliar and canker pathogens. *Phytophthora* species are associated with a range of tree decline syndromes throughout the world, which significantly affect natural ecosystems including Mediterranean ecosystems. These include *P. quercina* and *P. ramorum* on oak in Europe and North America, respectively. The most significant *Phytophthora* species in Australia associated with natural ecosystem decline is *P. cinnamomi,* identified as highly pathogenic on a wide host range of plants estimated at 2282 susceptible and 800 highly susceptible species, throughout the southwest of Western Australia. Based on the adverse role *Phytophthora* species play on the health of many ecosystems worldwide, it is not unreasonable to hypothesize that this new record may also be involved in *E. gomphocephala* decline.

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Table 1. Morphological characteristics of *Phytophthora* species isolated from declining *Eucalyptus gomphocephala* in the present study and the holotype *P. citricola* (Sawada 1927).

Character	*Phytophthora* isolate from *E. gomphocephala* decline	*P. citricola* holotype (Sawada 1927)
Oogonia diameter (µm)	(24-) 26 – 30 (-31)	(17-) 27 – 32 (-44)
Oospore diameter (µm)	(21-) 23 – 27	18 – 38
Oospore wall diameter (µm)	(1-) 2 – 3	1– 2
Antheridia length (µm)	(7-) 9 – 16 (-21)	12 – 13
Antheridia diameter (µm)	(6-) 8 – 11 (-12)	8 – 10
Sporangia length (µm)	(27-) 36 – 46 (-64)	21 – 70
Sporangia breadth (µm)	(18-) 20 – 29 (-34)	15 – 39
Papillae width (µm)	6 – 10	–
Papillae depth (µm)	1 – 2	< 3
Growth rate optimal	< 20°C	25 - 28°C

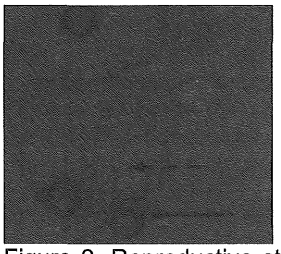

Figure 2. Reproductive structures of a *Phytophthora* species isolated from declining *E. gomphocephala*. Oogonia, oospores and antheridia (continuous arrows). Evacuated sporangia (dashed arrows) showing internal proliferation.