Age-related susceptibility of *Eucalyptus* species to *Phytophthora boodjera*

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Abstract

Phytophthora boodjera is a newly described pathogen causing damping-off and mortality of *Eucalyptus* seedlings in Western Australian (WA) nurseries. This study evaluated the age-related susceptibility of several taxa of mallee *Eucalyptus* to *P. boodjera* in sterilised washed river sand-infestation pot trials. *P. cinnamomi* and *P. arenaria* were included for comparison. *Eucalyptus* taxa were inoculated with individual *Phytophthora* isolates at 0, 2, 4, 12 and 88 week-old-seedlings. Pre-emergent mortality in the presence of *Phytophthora* was almost 100%. Post-emergent mortality was 50-100% depending on isolate compared to 0% for the control. Mortality was also high for inoculated 1-month old seedlings (46-68%) and root length of surviving seedlings was severely reduced. Death from root infection was not

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observed for seedlings inoculated at 12 and 88 weeks, but these developed root necrosis and reduced root dry weight compared to non-inoculated controls. *Phytophthora boodjera* is a pre- and post-emergent pathogen of mallee eucalypts. These eucalypts are susceptible to *P. boodjera* at all life stages tested, but the mortality rates declined with plant age. Similar results were obtained for *P. cinnamomi* and *P. arenaria.* The events leading to its recent appearance in the nurseries remain unknown and further investigations are underway to determine if this is an introduced or endemic pathogen. The approach used here to understand the impact of a *Phytophthora* species on multiple hosts at different seedling ages is novel and sets a benchmark for future work.

Introduction

Environmental conditions in nurseries such as warm temperature, over-watering, poor drainage and high seedling density are favorable to the development of disease epidemics caused by *Phytophthora* species (Old *et al*., 2003; Pérez-Sierra & Jung, 2013; Jung *et al.*, 2015; Prigigallo *et al*., 2015). Nursery propagation is a crucial stage of any *Eucalyptus* planting program and vulnerability to losses of planting stock due to pre- and post-emergence damping-off, or root and collar rot disease of older seedlings, can severely hamper their production (Gibson, 1975; Marks & Kassaby, 1976; Broembsen, 1984; Eldridge *et al*., 1994; Brown & Ferreira, 2000). *Phytophthora cinnamomi* and *P. cryptogea* have been reported as damping-off and root disease pathogens of *Eucalyptus* in nurseries (Hamm & Hansen, 1982). *Phytophthora cactorum*, *P. citricola* and *Pythium anandrum* caused a stem disease of various *Eucalyptus* species in Tasmanian nurseries (Wardlaw & Paizer, 1985).

Fungicide treatment regimes or sub-lethal inoculum levels can result in the presence of *Phytophthora* in asymptomatic nursery plants and as such these plants can be important

vectors of *Phytophthora* species that affect ornamental plants, agricultural crops, and forests (Brasier, 2008; Goss *et al*., 2011; Parke & Grünwald, 2012; Pérez-Sierra & Jung, 2013; Parke *et al*., 2014; Migliorini *et al*., 2015). There are numerous reports world-wide of cases where multiple *Phytophthora* species have been detected from both asymptomatic and symptomatic nursery plants or potting mix. More than 20 *Phytophthora* species were reported in Italian nurseries (Cacciola *et al*., 2008), and between 10 and 17 species of *Phytophthora* were detected in nurseries in Germany, Minnesota, California, and Spain (Themann *et al*., 2002; Schwingle *et al*., 2007; Moralejo *et al*., 2009; Yakabe *et al*., 2009; Bienapfl & Balci, 2014). Hardy & Sivasithamparam (1988) reported that eight species of *Phytophthora* including *P. drechsleri, P. nicotianae*, and *P. cactorum* were isolated from rotted roots of 65 plant taxa grown in containers in Western Australian (WA) nurseries. Davison *et al*. (2006) also obtained eight *Phytophthora* spp. from 10% of potting mix samples taken from 15 consignments of nursery-grown plants imported into WA from other states in Australia. A recent review based on data collected over 41 years by 38 research groups in 23 European countries documented 49 *Phytophthora* taxa in 670 nurseries (Jung *et al*., 2015).

Some exotic *Phytophthora* species have moved from nursery plants into forests, where they have caused epidemics in natural vegetation (Parke *et al*., 2014). For instance, in the USA, *P. lateralis* was first reported from horticultural nurseries in the Seattle area in the 1920s and spread to the native range of Port-Orford cedar (*Chamaecyparis lawsoniana*) in about 1950, where it was widely dispersed in rivers and along roadways (Hansen, 2011). *Phytophthora lateralis* is now causing disease outbreaks on *Chamaecyparis* trees in the landscapes of France and Scotland, where it has been traced to the outplanting of trees from infested nurseries (Robin *et al*., 2011; Brasier *et al*., 2012). Other examples of invasive forest pathogens that possibly have been introduced by the horticultural plant trade are *P.* x *alni* in Europe, *P. austrocedrae* in Chile, *P. kernoviae* in the United Kingdom, and *P. ramorum* in

Europe, North America and the UK (Brasier, 2008; Parke & Lucas, 2008; Parke & Grünwald, 2012). Jung *et al*. (2015) also reported the incidence of ubiquitous *Phytophthora* species in 732 European nurseries producing forest transplants, larger specimen trees, landscape plants and ornamentals, and in 2525 areas in which trees and shrubs were planted. They concluded that on average 3.6 *Phytophthora* species/taxa were isolated per infested nursery and planting site.

Since 2011, damping-off and seedling mortality were observed in nurseries producing mallee eucalypts (small trees that grow with multiple stems from underground lignituber) in large quantities for revegetation projects within the highly impacted wheat-belt region of Western Australia (WA). The causal agent of this disease was identified as *Phytophthora boodjera* based on a combination of morphology and a multi-gene phylogeny (Simamora *et al*., 2015). Symptoms associated with *P. boodjera* included stunted growth, which was often not observed until seedlings reached the 4-6 true leaf stage. As the season continued, seedling deaths were also observed especially along bench ridges. Losses due to this new disease were estimated at up to 40% on the worst-affected benches and the resulting short-fall in continuous supply of these seedlings was a concern for the nursery industry. In regards to the continued production of mallee eucalypts for restoration there are two main issues that must be addressed (i) is *P. boodjera* only a damping-off pathogen or can it infect and damage older seedlings and/or trees?, and (ii) is *P. boodjera* endemic to Western Australia? This study addresses the first of these questions by evaluating the age-related susceptibility of five taxa of mallee eucalypts to *P. boodjera*.

Materials and Methods

Biological materials

The age-related susceptibility to *P. boodjera* of five taxa of *mallee* eucalypts*; E. polybractea* (PLB), *E. kochii* subsp. *plenissima* (KOP), *E. kochii* subsp. *borealis* (KOB), *E. loxophleba* subsp. *lissophloia* (LOL), and two seedlots of *E. loxophleba* subsp. *gratiae* (LOG1 and LOG2) was tested in pot-trials. An isolate of *P. cinnamomi* was included for comparison in trials with 4 and 12 week-old seedlings several isolates of *P. arenaria* were also included (Table 1).

Inoculum preparation

400 mL of vermiculite substrate (1 L vermiculite, 10 g millet seeds and 600 mL V8 broth). was placed into each 500 mL Erlenmeyer flask, which was sealed with non-absorbent cotton wool and covered with aluminium foil. V8 broth consists of 0.1 L filtered V8 juice, 0.1 g $CaCO_3$, 0.9 L distilled water. The flasks were autoclaved three times at 121 ℃ for 20 minutes over three consecutive days, and then inoculated on the third day once the substrate had cooled. Inoculum per flask consisted of agar plugs (5 mm diameter) cut from a full of 7-day-old colony of a specific *Phytophthora* isolates grown on V8 agar (V8A same recipe as V8 broth with the addition of 17 g agar). Flasks were shaken and then placed inside zip-lock plastic bags and incubated at 20 ℃ in the dark. The flasks were shaken every 3 days in the first two weeks to evenly spread the inoculum. After incubation for five weeks, the inocula were rinsed with deionised water to remove excess nutrients (Matheron & Mircetich, 1985; Jung *et al*., 1996) immediately before sand infestation. Colonization of the inocula was confirmed by plating 3 g sub-samples onto NARPH agar, a *Phytophthora*-selective medium (Hüberli *et al*., 2000), and into a Petri dish containing deionized water. These were incubated

at room temperature and checked to ensure the viability of the inocula. The amount of inoculum used in all trials was 1 % of the weight of sand in the punnets and/or pots.

Experimental Design

Seeds, germinants, and 4-, 12- and 88-week-old *Eucalyptus* seedlings were tested for their age-related susceptibility toward *P. boodjera*, *P. arenaria* and *P. cinnamomi* in four separate experiments. All experiments were conducted under evaporatively-cooled glasshouse conditions (11-32 ˚C) in sand-infestation pot trials using sterilised washed river sand as the growth medium. The sand was steam sterilised in hessian bags in an aluminium box for at least two hours at 98 ˚C. Pots (150 mm, 1.9 L free-draining polyurethane pot) or punnets (Garden City Plastics, Canning Vale, WA, 90 mL per cell) were also sterilised before use. Pots and punnets were always arranged in a randomised complete block design on benches in the glasshouse. Plants were watered as required with deionised water to run-off and fertilized with water-soluble Thrive® (Yates Company, Australia). For each trial, the presence of *Phytophthora* in dead or diseased seedlings was confirmed by plating on NARPH the root collars and roots remaining at the end of the trial.

Pre-emergent damping-off

The first trial was conducted in sterilised 6-cell punnets using six host treatments (PLB, KOP, KOB, LOL, LOG1, LOG2). Each of the six cells in the punnet was sown with one of the six host treatments. Four *Phytophthora* isolates (Table 1) were each used to inoculated 6 separate replicate punnets and there were 6 non-inoculated control punnets (=30 punnets in total). In a second repeat trial, only four *Eucalyptus* host treatments were included (KOP, KOB, LOL, LOG1) and sterilised 4-cell punnets were used. Four *Phytophthora* isolates and one non-inoculated control were used in this six-replicate trial (=30 punnets in total).

Seeds of different host treatments were placed in random cells in each punnet. At the time of sowing, two grooves were made in the sand running the length of the punnet cell, one for placing the seeds and one for placing the vermiculite inoculum. In each cell, seeds (25) of each host treatment and vermiculite inocula (2.5 g) were placed directly into the grooves and covered with a thin layer of growth medium. Punnets were fertilised weekly with 2g/5 L of Thrive®. At day 14, the number of germinated seeds was counted. After 42 days (end of the trial) the number of surviving seedlings and seedling dry weight were recorded

Post-emergent damping-off

Experimental design was the same as for the pre-emergent damping-off trial except that instead of soil infestation at the time of seed sowing, infestation was done at 2 weeks after seed germination on day 14. A 10 mL sterile plastic tube was placed into the sand at time of sowing to keep a 5 mL space for the inoculum. Fourteen days later, the sand was inoculated by removing the plastic tubes and inserting 2.5 g of vermiculite inoculum into the holes, and covering the holes with sand. Punnets were watered, fertilized and assessed as per the per-emergent dampening of trial. The experiment was repeated as above using only four host treatments.

Susceptibility of 4-week-old seedlings

This trial was conducted in sterilised 6 cell punnets. Seeds of the six host treatments (PLB, KOP, KOB, LOL, LOG1, LOG2) were placed in random cells in each punnet. A 10 mL sterile plastic tube was placed into the sand at time of sowing to keep a 5 mL space for the inoculum. There were ten *Phytophthora* isolates and a non-inoculated control (Table 1), with five replicate punnets per isolate (=55 punnets). Seeds (25) of each host treatments were placed directly across the surface of the sand in the punnets. Four weeks after sowing (2 weeks after germination), the seedlings were inoculated with 2.5 g of vermiculite inoculum,

by placing the inoculum in a hole that was made at the time of sowing at one end of each cell. The holes were then covered with sand. Three days later the punnets were individually flooded in trays for 24 hours. The trays were filled with deionized water to the soil surface line. Thereafter, flooding was repeated weekly and seedlings were fertilised weekly with Thrive (2g/5 L).

Seedlings were harvested five weeks after inoculation (when 9 weeks old). The seedlings were removed from their punnets, sand was gently rinsed from the roots with tap water, and the seedlings were blotted dry with paper towels. Root length and seedling height of five seedlings selected at random for each treatment were measured and all five seedlings were put in a small paper envelope (50 x 80 mm), dried at room temperature for three weeks, and then weighed. Re-isolations were made from surface-sterilized root tissue plated on NARPH to confirm Koch's postulates for each treatment.

Susceptibility of 12-week-old seedlings

Seeds from six host treatments (PLB, KOP, KOB, LOL, LOG1, LOG2) were germinated in seedling trays containing sterilised washed river sand. Fourteen days after germination, individuals were pricked out into the six-cell punnets also containing sterilised washed river sand with one representative of each species per punnet. The seedlings were placed in cells in each punnet at random. Each punnet was considered a replicate. When pricking out, a sterile 10 mL plastic tube was inserted into the sand in each cell to retain the space for inserting the inoculum. Seedlings were fertilised weekly with 2g/5 L of Thrive®.

At 12 weeks (10 weeks after transferring to the punnets), the seedlings were inoculated with ten *Phytophthora* isolates (10 punnets per isolate) (Table 1). Control plants received sterile inoculum. There were 110 punnets in total. Sand was inoculated by removing the plastic tubes from each cell and placing 2.5 g of inoculum into each hole. The holes were then filled

with sand. Every two weeks the punnets were flooded with deionized water for 24 hours to stimulate the production of sporangia, zoospore release, and root infection by zoospores. Seedling were fertilized weekly with 5 g/5 L of Thrive® fertiliser. After one month, re-isolations from the flooding water were made by leaf baiting (*Pimelea ferruginea*, *Scholtzia involucrata*, *Hedera helix* (Ivy), *Eucalyptus seiberi* juvenile leaves) and leaves with lesions were plated onto NARPH to determine the viability of the inoculum.

Seedlings were harvested 12 weeks after inoculation. Before harvesting, seedling heights were measured. The seedlings were removed from their punnets, sand was gently rinsed from the roots with tap water, and the roots were blotted dry with paper towels. Re-isolations were made from surface-sterilized root tissue plated on NARPH to confirm Koch's postulates for each treatment. Roots and tops were oven dried at 60 ℃ for 7 days, and then weighed.

Susceptibility of 88-week-old seedlings

Eighty-week-old seedlings of 3 host treatments (PLB, KOP, LOG1) grown in 150 mm free-draining polyurethane pots were individually transferred into sterile 4 kg free-draining polyurethane pots (Garden City Plastics Canning Vale, WA) containing sterilised river sand as growing medium. At the time of potting up, two sterile polyurethane tubes (12.5 cm long and 2 cm diameter) were inserted into each pot, one at each side of the seedling. Seedlings were fertilised with 9 g/5 L Thrive® fertilizer at one-month intervals after potting.

After 8 weeks, the pots were inoculated with one of four isolates of *Phytophthora* (Table 1) by removing the polyurethane tubes and inserting the vermiculite inoculum (20 g) into each hole. The control treatment was given the same weight of non-inoculated vermiculite inoculum. The holes were then filled with growth medium. The experiment had six replicate pots for each combination . In order to stimulate the production of sporangia and the release

of zoospores from the inoculum source, the pots were flooded with deionised water for 24 hours on three occasions: immediately after inoculation, at day 14 and at day 28.

The time to seedling death post-inoculation was recorded and re-isolations were made from the root collar plated on NARPH to confirm Koch's postulates. Three months after inoculation, surviving seedlings were harvested. The stems were separated from the roots. Re-isolations were made from surface-sterilized root tissue plated on NARPH to confirm Koch's postulates for each treatment. Stem and leaves (tops) were bagged in separate paper bags for each plant, dried at 37 ℃ for 20 days and weighed.

Roots were washed carefully with tap water and blotted dry with paper towels. Whole root systems were visually rated for root rot on a scale 1 to 4 (1=no damage, 4=severe root damage). Roots and tops were dried at 60 ℃ for 7 days, and then weighed.

Data analysis

For each experiment, one-way ANOVA were performed in SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) to analyze the response of host treatments to infestation by *Phytophthora* isolates. Depending on the trial, the following variables were tested: the number of germinated seedlings, number of surviving seedlings, seedling dry weight, root length, seedling height, root dry weight and top dry weight. The means of the different treatments were compared using Duncan's multiple range test in SPSS.

Results

Pre-emergent damping-off

Seeds of all non-inoculated host treatments started to germinate on day 7 and by day 14 there was no further germination. The percentage of germination for all host treatments was >90 % on average (Table 2). For inoculated seeds, the germination was very poor or absent and the few emergent seedlings grew slowly, wilted and died within two weeks. In the second trial, after 14 days, the mean number of germinated seeds in inoculated cells was significantly ($P < 0.05$) lower than non-inoculated controls. Numbers of germinated seeds (from 25 seeds sown) ranged from 1.67 (± 0.9) to 7.67 (± 0.81) for inoculated seeds and 18.30 (±1.05) to 22.50 (± 0.70) for non-inoculated controls. After 42 days, in both trials, all *Phytophthora* isolates affected all host treatments, resulting in a significant ($P <0.05$) reduction in the number of germinants with less than one germinant surviving except for KOB and LOG1 inoculated with *P. cinnamomi* (Table 2).

Post-emergent damping-off

Seeds from all host treatments germinated between 7-14 days. After *Phytophthora* infestation, inoculated seedlings started to wilt and died within three weeks. After 42 days, the mean number of inoculated seedlings that survived was significantly ($P < 0.05$) lower than non-inoculated controls (Table 2). The average number of seedlings surviving ranged from 1.00 ± 0.47 for KOP inoculated with *P. boodjera* isolate PAB11.56 to 11.83 ± 1.14 for LOG1 inoculated with *P. cinnamomi* (Table 2). Seedling survival was very similar between the two trials.

Susceptibility of 4-week-old seedlings

Seeds germinated within fourteen days and the percentage of germination of each species varied between 75-90 %. Deaths of inoculated seedlings started at day 40, 12 days after inoculation. The number of seedlings that survived at the end of this trial varied between *Phytophthora* species (Table 3). Compared to the control non-inoculated seedlings, the percentage of seedlings surviving was 53.74 %, 67.7 5%, 43.13 % for *P. cinnamomi*, *P. arenaria*, and *P. boodjera*, respectively (Table 3).

Root systems of seedlings inoculated with *Phytophthora* had less root mass, shorter root length and necrotic lesions. In contrast, the roots in the control treatment were mostly white, long and healthy (Fig. 1). Total dry weight and root length for all host treatments inoculated with *Phytophthora* were significantly ($P<0.05$) correlated and only total dry weight is presented here. All *Phytophthora* isolates caused significant ($P <0.05$) reduction of total dry weight compared to controls in all host species (Fig. 2A). On average, *P. boodjera* caused the greatest reduction of total dry weight of all *Eucalyptus* species tested (60.96 % reduction in dry weight compared to non-inoculated controls), followed by *P. cinnamomi* (47.76 % reduction in dry weight) and *P. arenaria* (34.73 % reduction in dry weight). Of the *P. boodjera* isolates, VHS27382 and VHS27017 caused the greatest reduction in biomass, but were not significantly less than the other isolates.

Susceptibility of 12-week-old seedlings

No seedlings died during the trial, however, some seedlings developed foliar symptoms such as yellowing and loss of foliage by 10 weeks after inoculation. Nevertheless, this was not observed in all instances and the results were not quantified. Root systems of controls were healthier and larger than roots of inoculated seedlings (Fig. 3).

All *Phytophthora* isolates caused significant (P <0.05) reduction of root dry weight compared to controls for all host treatments except for PLB (Fig. 2B). On average, *P. boodjera* caused the greatest reduction of total root dry weight of all hosts treatments tested (42.12 % reduction in dry weight); *P. cinnamomi* (22.67 % reduction in dry weight) and *P. arenaria* (20.63 % reduction in dry weight). Of the *P. boodjera* isolates, VHS26806 and VHS27017 were the most pathogenic (Fig. 2B).

Susceptibility of 88-week-old seedlings

Eight seedlings died during this trial. The first plant death (KOP) occurred on day 18, 4 days after the second flooding, in a pot inoculated with *P. boodjera* isolate VHS27382. After the third flooding, one more seedling of KOP and one PLB infested with VHS 27382 died. *Phytophthora boodjera* isolate PAB 11.56 killed one seedling of LOG1, isolate VHS 26806 killed one seedling each of LOG1 and KOP, all after the third flooding. *Phytophthora cinnamomi* killed one seedling each of LOG1 and PLB, after the second and the third flooding, respectively. No non-inoculated control plants died. In all instances of plant mortality, *Phytophthora* was recovered from necrotic lesions at the collar (base of the stem) of the seedlings that had blocked the phloem at the base of the stem of the plants. The root systems appeared healthy.

Seedling height increase was greatest in non-inoculated control seedlings for all host treatments and the height increment was significantly (P < 0.05) less for inoculated seedlings (Table 4). There were negative correlations between root damage and height increment for KOP (r= -0.4539) and LOG 1 (r= -0.4387). Root disease scores were lowest in the non-inoculated controls and significantly (P <0.05) higher for inoculated seedlings (Fig. 4, Table 4).

There were significant (P< 0.05) differences in root dry weight between non-inoculated controls and inoculated seedlings with the exception of PLB and LOG1 inoculated with *P. cinnamomi* and PLB inoculated with *P. boodjera* isolate PAB11.56 (Fig. 5). On average, there was no significant (P<0.05) difference in pathogenicity of the isolates tested, however, *P. boodjera* isolate VHS27382 caused the greatest reduction in root dry weight (51.72 %) compared to VHS26806 (44.09 %), PAB11.56 (37.68 %) and *P. cinnamomi* isolate MU94-48 (33.64 %).

Reisolation of Phytophthora species

All *Phytophthora* isolates were consistently reisolated from the roots of inoculated seedlings of all ages. No Phytophthora species were isolated from the control seedlings and no control seedling showed wilting or died during any of the trials

Comparison of age classes

There was no analysis conducted to compare between the experiments with different aged hosts as designs were different and not all hosts or *Phytophthora* isolates were included in all experiments. However, there are qualitative observations that can be made. The host species tested were equally susceptible to damping-off of seeds and seedling for the three *P. boodjera* and one *P. cinnamomi* isolates tested. All hosts taxa inoculated at 4 and 12 weeks were more susceptible to *P. boodjera* than either *P. arenaria* or *P. cinnamomi.* Also for older hosts inoculated at 88 weeks, *P. boodjera* was more pathogenic than *P. cinnamomi*. Of the hosts tested at 4, 12 and 88 weeks, *E. polybracta* was less susceptible than other species.

Discussion

Phytophthora boodjera is clearly a pre- and post-emergent pathogen of mallee eucalypts, and it also infects the roots and reduces the growth of 4-, 12- and 88-week-old seedlings. These

eucalypts are susceptible to *P. boodjera* at all life stages tested, but death was confined to the pre- and post-emergent stages with specific exceptions that are addressed below. One isolates of *P. cinnamomi* was included for comparison in all trials and hosts followed the same pattern of age-related susceptibility. *Phytophthora arenaria* was included in the trials for two of the age classes. In each case it was less pathogenic than *P. boodjera*.

In this study, *P. boodjera, P. arenaria* and *P. cinnamomi* were all associated with damping-off of different *Eucalyptus* seedlings following inoculation between age 0 and 4 weeks. Mortality was highest when inoculated pre-emergence, but was still over 60 % when inoculated 2 and 4 weeks post emergence and if the surviving seedlings had been left for longer, given the observed level of damage to their roots, it is expected that they would have died. Seedlings inoculated at 12 and 88 weeks did not die from root infection. Regardless of age, *P. boodjera* was more aggressive to eucalypt seedlings than *P. arenaria* and *P. cinnamomi*.

Several seedlings in the 88-week-old trial died; however, these deaths were due to collar rot as a consequence of flooding and collar infection, not from root infection. When these seedlings were excluded from the final data set, root infection reduced seedling growth to a similar extent to that observed for 12-week-old seedlings and less than that of observed for 4-week-old seedlings. The death of seedlings at this age by collar rot is, however, very important, as extreme weather events resulting in flooding could potentially result in seedling and/or tree death in environmental plantings and neighboring natural stands if *P. boodjera* is present. Such weather events are predicted to increase under future climate change scenarios for the south-west region of WA (Silberstein *et al.*, 2012)

Endemic *Phytophthora* species in their natural ecosystems will have coevolved with their hosts, and are not expected to cause severe disease. In forests and woodlands, the

interrelationship between trees and their endemic pests and pathogens, while dynamic and complex, are usually not threatening to the forest ecosystems (Liebhold *et al*., 2012). When conditions change, however, through the transport of pathogens or trees to new environments (Brasier, 2008; Callaghan & Guest, 2015) or exposure to new hosts lacking coevolved resistance, or modification in the environment itself, forest pathogen dynamics alter, often to the detriment of the forest. So although formerly benign, the pathogen could later develop as aggressive and destructive (Hansen, 2015). The emergence of new species of pathogens can result in new disease epidemics that can be ecologically and economically difficult to manage, and this also has serious implications for biosecurity (Scott *et al*., 2013; Ik-Hwa & Choi, 2014). *Phytophthora boodjera* is a pathogen of young mallee eucalypts commonly used in environmental restoration and while predominantly a damping-off pathogen, it reduces the growth and vigour of older seedlings and can cause mortalities after flooding. Currently, it is not known if this species is endemic to the region, or introduced.

Phytophthora boodjera is most closely related to *P. arenaria* and *P. alticola* nom. dub. *Phytophthora arenaria* was mainly isolated from roots of dead and dying *Banksia* species and from the rhizosphere soil of those species (Rea *et al*., 2011). Although only described in 2011, *P. arenaria* isolates have been recovered since 1986 in WA. In South Africa, *P. alticola* nom. dub. was isolated only from eucalypt plantations (Maseko *et al*., 2007) and not from natural environments (Nagel *et al*., 2013; Oh *et al*., 2013), and is considered to be introduced, probably originating in Australia due to its association to *Eucalyptus*. *Phytophthora boodjera* has recently emerged as a pathogen in some WA plant production nurseries and is now frequently isolated from disturbed environments. However, it has been recovered infrequently from natural ecosystems in WA, despite extensive sampling in the region (Burgess *et al*., 2009; Rea *et al*., 2011). Thus, while *P. arenaria* is considered endemic to WA, the origin of *P. boodjera* is currently uncertain.

If it is endemic, the role of *P. boodjera* as a damping-off pathogen in natural ecosystems could be as an agent of negative density dependence (Bagchi *et al*., 2014; Laliberté *et al*., 2015). Inoculum of soil-borne pathogens builds up in the root zone of mature plants, resulting in low conspecific seedling existence and growth. Recruits function better and attain higher densities away from conspecific mature individuals, where pathogen inoculum is lower (Laliberté *et al*., 2015).

Phytophthora boodjera has only recently been found in WA and has mostly been isolated from dead and dying eucalypt seedlings in plant production nurseries and from declining trees (predominantly *Myrtaceae*) in disturbed urban landscapes, and once from *Xanthorrhoea preissii*. Even though *P. boodjera* is unlikely to kill older seedlings or trees, as a damping-off pathogen it could affect regeneration and seedling recruitment. This condition may not be readily noticed during normal observation of vegetation health for many years, but will cause important changes in the flora species composition of the ecosystem.

When forest trees are grown in nurseries, depending on hygiene practice, they can be exposed to many diseases, including *Phytophthora* root rots. *Phytophthora* species commonly involved in forest nurseries are often the same species affecting agricultural commodities in the area (Hansen, 2008; Jung *et al.*, 2015). However, some *Phytophthora* found frequently in nurseries, such as *P. cactorum*, are rarely found in forest settings, while *P. foliorum* and *P. hedraiandra* have only been found in nurseries. On the other hand, *P. quercetorum* and *P. siskiyouensis* were only recovered from forests, and have yet to be discovered in nurseries. Perhaps these *Phytophthora* need very specific environmental conditions and are host specific (Balci & Bienapfl, 2013). *Phytophthora cinnamomi, P. cryptogea and P. citricola sensu lato* have been reported as damping-off and root disease pathogens of *Eucalyptus* in nurseries (Hamm & Hansen, 1982; Wardlaw & Paizer, 1985). Many soilborne species of

Phytophthora have been isolated from eucalypt forests but only *P. cinnamomi* and *P. multivora* have been associated with major damage to the forests (Shearer & Smith, 2000; Scott *et al*., 2009).

In the past seven years, 13 new *Phytophthora* species have been described from Western Australia and at this stage very little is known about these species, including *P. boodjera.* Studies have only just begun on determining the origin, biology, epidemiology and host range of these species. Currently, while *P. boodjera* is a pathogen of mallee eucalypts, the events leading to its recent appearance in the WA nurseries and its origin remain unknown. As *P. boodjera*, besides being a damping off pathogen, has the ability to impact the growth of older plants the consequences of its introduction to natural ecosystems are also unknown. Thus, the precautionary principle should be invoked by land management groups not wishing to introduce an unknown pathogen into their environmental plantings. Further work on the distribution of *P. boodjera* within the landscape, its epidemiology and management within the nursery are currently underway.

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References

Bagchi R, Gallery RE, Gripenberg S, *et al.*, 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature* **506**, 85-8.

Balci Y, Bienapfl JC, 2013. *Phytophthora* in US forests. In: Lamour K, ed. *Phytophthora: a global perspective.* Wallingford: CAB International 135–45.

Bienapfl JC, Balci Y, 2014. Movement of *Phytophthora* spp. in Maryland's nursery trade. *Plant Disease* **98**, 133-44.

Bown BN, Ferreira FA, 2000. Disease during propagation of eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN, eds. *Diseases and Pathogens of Eucalypts.* Collingwood: CSIRO Publishing, 119-51.

Brasier CM, 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* **57**, 792-808.

Brasier CM, Franceschini S, Vettraino A, *et al.*, 2012. Four phenotypically and phylogenetically distinct lineages in *Phytophthora lateralis*. *Fungal Biology* **116**, 1232-49.

Broembsen SLV, 1984. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. *Phytophylactica* **16**, 221-5.

Burgess TI, Webster JL, Ciampini JA, White D, Hardy GESJ, Stukely MJC, 2009. Re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in Western Australia using molecular techniques. *Plant Disease* **93**, 215-23.

Cacciola SO, Pane A, Martini P, Agosteo GE, Raudino F, Lio GMDS, 2008. Recovery of *Phytophthora* species from potted ornamentals in commercial nurseries in Italy. *Journal of Plant Pathology* **90**, S2. 185.

Callaghan S, Guest D, 2015. Globalisation, the founder effect, hybrid *Phytophthora* species and rapid evolution: new headaches for biosecurity. *Australasian Plant Pathology* **44**, 255-62.

Davison EM, Drenth A, Kumar S, Mack S, Mackie AE, McKirdy S, 2006. Pathogens associated with nursery plants imported into Western Australia. *Australasian Plant Pathology* **35**, 473-5.

Eldridge K, Davison J, Harwood C, Wyk GV, 1994. *Eucalypt domestication and breeding*. New York: Oxford University Press.

Gibson IAS, ed, 1975. *Diseases of forest trees planted as exotics in the Tropics and Southern Hemisphere. Part I. Important members of the Myrtaceae, Leguminosae, Verbenaceae and Meliaceae,*. Oxford: Commonwealth Mycological Institute: Kew and Commonwealth Forestry Institute.

Goss EM, Larsen M, Vercauteren A, Werres S, Heungens K, Grünwald NJ, 2011. *Phytophthora ramorum* in Canada: Evidence for migration within North America and from Europe. *Phytopathology* **101**, 166-71.

Hamm PB, Hansen EM, 1982. Pathogenicity of *Phytophthora* species to Pacific Northwest USA conifers. *European Journal of Forest Pathology* **12**, 167-74.

Hansen EM, 2008. Alien forest pathogens: *Phytophthora* species are changing world forests. *Boreal environment research* **13**, 33–41.

Hansen EM, 2011. *Phytophthora lateralis*. Forest Phytophthoras 1. http://journals.oregondigital.org/ForestPhytophthora/article/view/1816.

Hansen EM, 2015. *Phytophthora* species emerging as pathogens of forest trees. *Current Forestry Report* **1**, 16-24.

Hardy GESJ, Sivasithamparam K, 1988. *Phytophthora* spp. associated with container-grown plants in nurseries in Western Australia. *Plant Disease* **72**, 435-7.

Hüberli D, Tommerup IC, Hardy GESJ, 2000. False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology* **29**, 164–9.

Ik-Hwa H, Choi W, 2014. *Phytophthora* species, new threats to plant health in Korea. *Plant Pathology* **30**, 331-42.

Jung T, Blaschke M, Neumann P, 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology* **26**, 253–72.

Jung T, Orlikowski L, Henricot B, *et al.*, 2015. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of Phytophthora diseases. *Forest Pathology*. http://onlinelibrary.wiley.com/doi/10.1111/efp.12239/epdf.

Laliberté E, Lambers H, Burgess TI, Wright J, 2015. Phosporus limitation, soil borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytologist* **206**, 507-21.

Liebhold AM, Brockerhoff EG, Garret LJ, Parke JL, Britton KO, 2012. Live Plant imports: the major pathway for forest insect and pathogen invasion of the US. *Frontiers in Ecology and the Environment* **10**, 135-43.

Marks GC, Kassaby FY, 1976. Pathogenicity of nine species of *Phytophthora* to *Eucalyptus sieberi*. *Australian Forest Research* **7**, 59-63.

Maseko B, Burgess TI, Coutinho TA, Wingfield MJ, 2007. Two new *Phytophthora* species from South African *Eucalyptus* plantations. *Mycological Research* **111**, 1321-38.

Matheron ME, Mircetich SM, 1985. Pathogenicity and relative virulence of *Phytophthora* spp. from walnut and other plants to rootstocks of English walnut trees. *Phytopathology* **75**, 977-81.

Migliorini D, Ghelardini L, Tondini E, Luchi N, Santini A, 2015. The potential of symptomless potted plants for carrying invasive soilborne plant pathogens. *Diversity and Distributions* **21**, 1218-29.

Moralejo E, Perez-Siera AM, Álvarez LA, Belbahri L, Lefort F, Descals E, 2009. Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain. *Plant Pathology* **58**, 100-10.

Nagel JH, Gryzenhout M, Slippers B, Wingfield MJ, 2013. The occurrence and impact of *Phytophthora* on the African continent. In: Lamour K, ed. *Phytophthora: a global perspective.* Wallingford: CAB International, 204–14.

Oh E, Gryzenhout M, Wingfield WD, Wingfield MJ, Burgess TI, 2013. Surveys of soil and water reveal a goldmine of *Phytophthora* diversity in South African ecosystems. *IMA Fungus* **4**, 123-31.

Old KM, Wingfield MJ, Yuan ZQ, 2003. *A manual of diseases of eucalypts in South-East Asia.* Bogor, Indonesia: Center for International Forestry Research.

Parke JL, Grünwald NJ, 2012. A Systems Approach for Management of Pests and Pathogens of Nursery Crops. *Plant Disease* **96**, 1236-44.

Parke JL, Knaus BJ, Fieland VJ, Lewis C, Grünwald NJ, 2014. *Phytophthora* community structure analyses in Oregon nurseries inform systems approaches to disease management. *Phytopathology* **104**, 1052-62.

Parke JL, Lucas S, 2008. Sudden oak death and ramorum blight. *The Plant Health Instructor*. DOI. 10.1094/PHI-I-2008-0227-01. http://www.apsnet.org/edcenter/intropp/lessons/fungi/oomycetes/Pages/SuddenOakDeath.aspx.

Pérez-Sierra A, Jung T, 2013. *Phytophthora* in woody ornamental nurseries. In: Lamour K, ed. *Phytophthora: a global perspective.* Wallingford: CAB International, 166-77.

Prigigallo MI, Mosca S, Cacciola SO, Cooke DEL, Schena L, 2015. Molecular analysis of *Phytophthora* diversity in nursery-grown ornamental and fruit plants. *Plant Pathology* **64**, 1308-19.

Rea AJ, Burgess TI, Hardy GESJ, Stukely MJC, Jung T, 2011. Two novel and potentially endemic species of *Phytophthora* associated with episodic dieback of Kwongan vegetation in the south-west of Western Australia. *Plant Pathology* **60**, 1055-68.

Robin C, Piou D, Feau N, Douzon G, Schenck N, Hansen EM, 2011. Root and aerial infections of *Chamaecyparis lawsoniana* by *Phytophthora lateralis*: A new threat for European countries. *Forest Pathology* **41**, 417- 24.

Schwingle BW, Smith JA, Blanchette RA, 2007. *Phytophthora* species associated with diseased woody ornamentals in Minnesota nurseries. *Plant Disease* **91**, 97-102.

Scott PM, Burgess TI, Barber PA, *et al.*, 2009. *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia* **22**, 1-13.

Scott PM, Burgess TI, Hardy GESJ, 2013. Globalization and *Phytophthora*. In: Lamour K, ed. *Phytophthora: a global perspective.* Wallingford: CAB International, 226–32.

Shearer BL, Smith IW, 2000. Disease of eucalypts caused by soilborne species of *Phytophthora* and *Pythium*. In: Keane PJ, Kile GA, Podger FD, Brown BN, eds. *Diseases and pathogens of eucalypts.* Collingwood: CSIRO Publishing, 259-91.

Simamora AV, Stukely MJ, Hardy GESJ, Burgess TI, 2015. *Phytophthora boodjera* sp.nov., a damping-off pathogen in production nurseries and from urban and natural landscapes, with an update status of *P. alticola*. *IMA FUNGUS* **6**, 319-35.

Silberstein RP, Aryal SK, Durrant J, Pearcey M, Braccia M, Charles SP, *et al.* 2012. Climate change and runoff in south-western Australia. *Journal of Hydrology* 475: 441-455.

Themann K, Werres S, Lüttmann R, Diener HA, 2002. Observation of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock. *European Journal of Plant Pathology* **108**, 337–43.

Wardlaw TJ, Paizer C, 1985. Stem diseases in nursery seedlings caused by *Phytophthora cactorum*, *P. citricola* and *Pythium anandrum*. *Australasian Plant Pathology* **14**, 57-9.

Yakabe LE, Blomquist CL, Thomas SL, Macdonald JD, 2009. Identifiaction and frequency of *Phytophthora* species associated with foliar diseses in California ornamental nurseries. *Plant Diseases* **93**, 884-90.

Figure Captions

Figure 1. Comparison of root length and damage of (A) *Eucalyptus kochii* subsp. *plenissima* (B) *E. kochii* subsp. *borealis* and (C) *E. loxophleba* subsp. *gratiae* five weeks after inoculation with (left to right) non-inoculated control, *Phytophthora cinnamomi* MP94-48, *P. arenaria* ENA1, *P. boodjera* VHS26806 and VHS27382. Seedlings were inoculated four weeks after sowing.

Figure 2. (A) Mean total dry weight (± standard error) of *Eucalyptus* seedlings inoculated with *Phytopthora cinnamomi* (blue), *P. arenaria* (red) and *P. boodjera* (green) isolates at 4 weeks and (B) Mean root dry weight (± standard error) for *Eucalyptus* seedlings inoculated at 12 weeks after sowing. For each host-isolate combination, different letters indicate significant ($P<0.05$) differences according to Duncan's multiple range test.

Figure 3. Comparison of size and root damage of (A) *Eucalyptus kochii* subsp. *plenissima* (B) *E. loxophleba* subsp. *lissopholia* and (C) *E. loxophleba* subsp. *gratiae* 12 weeks after inoculation; (left to right) non-inoculated control, *P. cinnamomi* MP94-48, *P. arenaria* ENA1, *P. boodjera* VHS26806 and VHS27382. Seedlings were inoculated 12 weeks after sowing.

Figure 4. Root reduction of (A) *Eucalyptus loxophleba* subsp. *gratiae*, (B) *E. kochii* subsp. *plenissima* and (C) *E. polybracta* inoculated with *Phytophthora* when 88 weeks old. Whole root systems were visually rated for root rot on a scale of 1 to 4 (1=no damage, 4=severe root damage). All control plants had a rating of 1.

Figure 5. Mean root dry weight (± standard error) of *Eucalyptus* seedlings inoculated with *Phytopthora cinnamomi* (blue) and *P. boodjera* (green) isolates at 88 weeks after sowing. For each host-isolate combination, different letters indicate significant ($P<0.05$) differences according to Duncan's multiple range test.

Table 1. *Phytophthora* isolates and species used in the pathogenicity trials.

Isolate	Identity	Host	Location	Seedling Age (weeks)				
				0	2	4	12	88
VHS 26806 CBS 138637	*P. boodjera*	soil	Tincurrin, WA	✔	✔	✔	✔	✔
VHS 26631	*P. boodjera*	*Eucalyptus* sp.	Kensington, Perth, WA			✔	✔	
VHS 27017	*P. boodjera*	*Eucalyptus* sp.	Tincurrin, WA			✔	✔	
VHS 27382	*P. boodjera*	*Xanthorrhoea preissii*	Stirling, Perth, WA	✔	✔	✔	✔	✔
PAB 11.56	*P. boodjera*	*Agonis flexuosa*	Mt Claremont, Perth, WA	✔	✔	✔	✔	✔
PAB 11.67	*P. boodjera*	*E. marginata*	Dalkeith, Perth, WA			✔	✔	
ENA 1 CBS 125800	*P. arenaria*	*E. drummondii*	Eneabba, WA			✔	✔	
ENA 3 CBS127950	*P. arenaria*	*E. drummondii*	Eneabba, WA			✔	✔	
VHS 25370	*P. arenaria*	*Banksia* sp.	Ellenbrook, Perth, WA			✔	✔	
MP 94-48	*P. cinnamomi*	*E. marginata*	Willowdale, WA	✔	✔	✔	✔	✔

Table 2. Mean number of surviving germinants at 42 days (also at 14 days for Trial 2) of *Eucalyptus kochii* ssp. *plenissima* (KOP), *E. kochii* ssp. *borealis* (KOB), *E. loxophleba* ssp. *lissophloia* (LOL), *E. polybractea* (PLB), and two seedlots of *E. loxophleba* ssp. *gratiae* (LOG1 and LOG2) inoculated either pre- or post-emergence with three isolates of *P. boodjera* (PAB 11.56, VHS26806 and VHS 27382) and one *P. cinnamomi* isolate (MP 94-48). Twenty five seeds of each host taxa were sown.

***Phytophthora* isolate**	**Number of surviving germinants**					
	Pre-emergent inoculation			**Post-emergent Inoculation**		
	Trial 1	**Trial 2**		**Trial 1**	**Trial 2**	
	42 days	**14 days**	**42 days**	**42 days**	**14 days**	**42 days**
***Eucalyptus kochii* subsp. *plenissima* (KOP)**						
PAB 11.56	0.00 a[1]	5.50 a	0.17 a	6.8 3 a	19.00 a	1.00 a
VHS 26806	0.00 a	3.33 a	0.00 a	4.67 a	21.17 a	2.67 a
VHS 27382	0.00 a	2.00 a	0.33 a	4.17 a	21.00 a	1.50 a
P. cinnamomi	0.00 a	2.33 a	0.33 a	5.17 a	20.00 a	6.00 b
control	10.00 b	18.3 b	18.33 b	10.83 b	19.33 a	19.33 c
***Eucalyptus kochii* subsp. *borealis* (KOB)**						
PAB 11.56	0.00 a	4.50 a	0.33 a	7.50 a	20.17 a	7.33 a
VHS 26806	0.0 0 a	3.00 a	0.33 a	6.67 a	18.00 a	7.67 a
VHS 27382	0.00 a	1.67 a	0.00 a	6.00 a	18.83 a	8.17 a
P. cinnamomi	0.00 a	4.00 a	1.83 b	10.67 a	21.50 a	10.50 a
control	10.33 b	22.50 b	21.83 c	16.33 b	21.67 a	20.83 b
***Eucalyptus loxophleba* subsp. *lissophloia* (LOL)**						
PAB 11.56	0.00 a	5.00 a	0.00 a	3.33 a	18.83 a	5.50 a
VHS 26806	0.17 a	4.50 a	1.00 a	4.17 a	21.33 a	3.50 a
VHS 27382	0.17 a	6.00 a	0.17 a	2.83 a	20.67 a	2.83 a
P. cinnamomi	0.17 a	3.67 a	0.33 a	3.67 a	20.83 a	6.67 a
control	12.33 b	19.83 b	19.33 b	10.83 b	19.50 a	19.17 b
***Eucalyptus polybractea* (PLB)**						
PAB 11.56	0.00 a			5.17 a		
VHS 26806	0.00 a			2.67 a		
VHS 27382	0.17 a			4.33 a		
P. cinnamomi	0.67 a			2.67 a		
control	9.67 b			11.50 b		
***Eucalyptus loxophleba* subsp. *gratiae* 1 (LOG1)**						
PAB 11.56	0.00 a	4.50 ab	0.17 a	5.00 a	20.33 a	6.50 a
VHS 26806	0.00 a	7.33 b	0.50 a	3.67 a	21.17 a	8.00 a
VHS 27382	0.00 a	4.67 ab	0.83 a	2.33 a	21.83 a	5.17 a
P. cinnamomi	0.17 a	7.67 b	2.50 b	2.83 a	21.17 a	11.83 b
control	16.50 b	20.00 c	19.83 c	17.83 b	20.67 a	21.67 c
***Eucalyptus loxophleba* subsp. *gratiae* 2 (LOG2)**						
PAB 11.56	0.0 0 a			4.00 a		
VHS 26806	0.00 a			3.33 a		
VHS 27382	0.00 a			3.17 a		
P. cinnamomi	0.83 a			3.17 a		
control	11.33 b			12.17 b		

[1]Numbers within a column followed by different letters are significantly ($P < 0.05$) different according to Duncan's multiple range test.

Table 3. Mean number of surviving germinants of *Eucalyptus kochii* ssp. *plenissima* (KOP), *E*. *kochii* ssp. *borealis* (KOB), *E*. *loxophleba* ssp. *lissophloia* (LOL), *E. polybractea* (PLB), and two seedlots of *E*. *loxophleba* ssp. *gratiae* (LOG1 and LOG2) inoculated at week 4 with different six isolates of *P. boodjera,* three of *P. arenaria* and one *P. cinnamomi* isolate. Survival determined 5 weeks after inoculation. Twenty five seeds of each host taxa were sown.

***Phytophthora* Isolate**	**Number of surviving germinants**						**MEAN**
	KOP	**KOB**	**LOL**	**PLB**	**LOG1**	**LOG2**	
Control	20.40 e	22.20 f	22.80 d	21.40 e[1]	21.00 e	22.20 d	21.67
P. cinnamomi							
MP 98-48	10.60 abc	13.20 bcde	9.80 ab	10.20 abcd	12.60 b	13.40 abc	11.63
P. arenaria							
ENA 3	11.00 abc	16.00 de	11.80 b	11.40 abcd	13.00 b	13.60 abc	12.80
ENA 1	15.80 d	18.80 def	12.40 b	14.60 cd	14.80 c	16.20 c	15.43
VHS 25370	17.20 de	19.00 ef	15.00 c	12.80 c	16.00 d	15.60 bc	15.93
P. boodjera							
VHS 26631	12.80 bcd	14.00 cde	11.20 b	8.80 ab	9.40 ab	9.40 ab	10.93
PAB 11.67	12.20 c	12.60 abcd	11.40 b	15.80 d	9.00 ab	9.60 ab	11.77
VHS 27382	8.80 ab	8.60 abc	7.60a	7.00 a	6.20 a	7.20 a	7.56
VHS 26806	8.20 ab	13.80 bcde	8.80 a	9.40 b	6.20 a	10.60 abc	9.50
PAB 11.56	7.80 ab	8.60 abc	8.40 a	9.20 ab	7.00 a	9.80 ab	8.46
VHS 27017	6.40 a	6.60 a	7.00 a	6.00 a	5.80 a	10.20 b	7.00

[1]Numbers within a column followed by different letters are significantly (P<0.05) different according to Duncan's multiple range test.

Table 4. Mean height increment and root damage of 88-week-old seedlings of *Eucalyptus polybracta* (PLB) *E. kochii* subsp. *plenissima* (KOP), *E. loxophlebai* subsp. *gratiae* LOG1) inoculated with different *Phytophthora* species and isolates. Whole root systems were visually rated for root rot on a scale 1 to 4 (1= no damage, 4= severe root damage).

	PLB		**KOP**		**LOG1**	
***Phytophthora* Isolate**	Height increment (cm)	Root damage score	Height increment (cm)	Root damage score	Height increment (cm)	Root damage score
Control	25.50 b[1]	1.33 a	33.58 b	1.83 a	17.00 c	1.17 a
P. cinnamomi						
MP 98-48	19.43 ab	2.83 b	14.50 a	2.67 ab	11.25 b	3.00 b
P. boodjera						
PAB 11.56	19.08 ab	2.17 ab	11.50 a	3.83 c	12.67 bc	3.17 b
VHS 26806	15.17 a	3.00 b	10.17 a	3.33 bc	4.25 a	3.83 b
VHS 27382	16.50 a	2.83 b	12.33 a	3.17 bc	8.67 ab	3.33 b

[1]Numbers within a column followed by different letters are significantly (P<0.05) different according to Duncan's multiple range test.