The susceptibility to *Phytophthora cinnamomi* of the threatened species:

- **Boronia gunnii**
- **Boronia hemichiton**
- **Boronia hippopala**
- **Philotheca freyciana**

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Department of Primary Industries and Water
Acknowledgments

Personnel with the Threatened Species Section (TSS) and the Royal Tasmanian Botanical Gardens (RTBG) collected cuttings of the four target species from wild populations, while RTBG personnel propagated the plants required for the *Phytophthora cinnamomi* trials — particular thanks go to Natalie Tapson and Lorraine Perrins. Diana Munro and Wayne Williams of DPIW’s Biosecurity & Product Integrity Unit provided assistance with managing the glasshouse and watering regime at the New Town Laboratories, while Chang-You Pan and Ziqing Yuan provided laboratory assistance.

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEPHA</td>
<td>Tasmanian Department of Environment, Parks, Heritage and the Arts</td>
</tr>
<tr>
<td>DEWHA</td>
<td>Australian Department of Environment, Water, Heritage and the Arts</td>
</tr>
<tr>
<td>DPIW</td>
<td>Tasmanian Department of Primary Industries and Water</td>
</tr>
<tr>
<td>EPBC Act</td>
<td>Commonwealth <em>Environment Protection and Biodiversity Conservation Act 1999</em></td>
</tr>
<tr>
<td>NRM Region</td>
<td>Natural Resource Management Region</td>
</tr>
<tr>
<td>RTBG</td>
<td>Royal Tasmanian Botanical Gardens (DEPHA)</td>
</tr>
<tr>
<td>TSP Act</td>
<td>Tasmanian <em>Threatened Species Protection Act 1995</em></td>
</tr>
<tr>
<td>TSS</td>
<td>Threatened Species Section (DPIW)</td>
</tr>
</tbody>
</table>

Plant nomenclature follows Buchanan (2007) except where otherwise noted.
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BACKGROUND

Phytophthora cinnamomi (Rands) is an introduced plant pathogen that can cause disease in plants in buttongrass moorland, heathland and dry sclerophyll communities in Tasmania (Podger et al. 1990a). It lives within the root systems of host species and may cause extensive root rot, killing the more susceptible plant species. A number of genera and species from the Rutaceae family are known to be hosts to P. cinnamomi, among them Boronia citriodora, Boronia pilosa, Correa reflexa, Nematolepis squamea and Philotheca virgata (Podger et al. 1990a), while for others there is proven pathogenicity (glasshouse or field), viz., Boronia pilosa and Nematolepis squamea (Podger et al. 1990a) and Phebalium daviesii (Barker & Wardlaw 1995).

Recent revisions in the taxonomy of the Rutaceae family have resulted in the description of new species in the Philotheca and Boronia genera in Tasmania (Rozefelds 2001; Duretto 2003). Four of these species (all Tasmanian endemics) — Boronia gunnii, Boronia hemichiton, Boronia hoppopala and Philotheca freyciana — are now listed on the Tasmanian Threatened Species Protection Act 1995 and the Commonwealth Environment Protection and Biodiversity Conservation Act 1999. The EPBC listing advice for each species cited infection by Phytophthora cinnamomi as a potential threat (see DEWHA website), with a range of recovery actions proffered to mitigate the impact of the disease (following Schahinger 2004). Recognition of the potential threat of P. cinnamomi to each of the four species was considered a necessary precaution given the known susceptibility of other species in the Rutaceae family and the distribution of the species within the disease’s climatic domain for P. cinnamomi expression (Podger et al. 1990b).

The ‘Strategic Regional Plan for Tasmania, Conservation of Tasmanian Plant Species & Communities Threatened by Phytophthora cinnamomi’ (Schahinger et al. 2003) aims to establish representative management areas for the protection of threatened species at risk from P. cinnamomi. Changes in the listing of threatened species requires a reassessment of those species listed for management under this plan, the initial step for newly-listed species being the assessment of their susceptibility to P. cinnamomi. This research project will undertake susceptibility testing for the four aforementioned species: Boronia gunnii, Boronia hemichiton, Boronia hoppopala and Philotheca freyciana. The results from this study will also inform the risk assessment underlying the threatened status of each species.

AIM

The aim of the study is to assess the susceptibility to Phytophthora cinnamomi of the following plant species under glasshouse conditions:

- Boronia gunnii Hook f.
- Boronia hemichiton Duretto
- Boronia hoppopala Duretto
- Philotheca freyciana Rozefelds

METHODOLOGY

Cuttings of each of the target species were obtained from wild populations (Appendix 1). Plants for the Phytophthora cinnamomi susceptibility trials were propagated by the Royal Tasmanian Botanical Gardens in a lightly fertilised potting mix (Appendix 2). Plants of the known P. cinnamomi susceptible species Aotus ericoides and the known P. cinnamomi resistant species Leptospermum scoparium were obtained from a commercial nursery. The age of plants tested varied from 4 years for the Boronia species and 3–7 years for Philotheca freyciana. The age of the commercially obtained plants was unknown.

The available plants were divided between treatment (Phytophthora cinnamomi inoculation), and control (sterile inoculation), and grouped into two separated trays (Plate 1; Table 1). A random block layout of plants was used in each tray. The number of plants available for testing was limited for most of the threatened species due to a lack of plants in propagation. Plant origins, pot sizes and treatment allocations are detailed in Appendix 1. Plants were placed in a glasshouse at DPIW’s New Town Laboratories on 11 May 2007, six months prior to the inoculation process.

All potted plants were tested for infection by Phytophthora cinnamomi on introduction to the glasshouse. Plants were placed in an undrained tray and flood irrigated with water. Lupin baits were
Phytophthora cinnamomi susceptibility trials 2007–2008

floated in the trays around the pots and plated using the technique of Ribeiro (1978) to isolate P. cinnamomi.

Table 1. Plants used in the Phytophthora cinnamomi susceptibility trials.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boronia gunnii</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Boronia hemichiton</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Boronia hippopala</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Philotheca freyciana</td>
<td>12</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Aotus ericoides (susceptible control)</td>
<td>17</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Leptospermum scoparium (resistant control)</td>
<td>17</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>23</td>
<td>42</td>
</tr>
</tbody>
</table>


Inoculation

Phytophthora cinnamomi inoculum was produced from a fresh isolate obtained from soil collected at the base of a dying Xanthorrhoea australis (southern grasstree) growing in the southeastern corner of Douglas-Apsley National Park, in the general region of the target species.

Inoculum was prepared in the laboratory as a V8® juice growth medium according to the method of Ribeiro (1978). Each treatment plant was inoculated by gently opening a hole in the soil and pouring in 10 ml of inoculum broth. Control plants were inoculated with 10 ml of the V8® juice broth without Phytophthora cinnamomi. The first inoculation occurred on 26 November 2007, and a second inoculation was conducted on 10 December 2007.

Glasshouse temperature was maintained around 20° C and not less than 15°C or higher than 30°C. Plants were watered by hand at regular intervals during the trial.
**Monitoring health and isolation of Phytophthora cinnamomi**

The plants were scored for health approximately every two weeks during the trial using the scale presented in Table 2. Any plants that had lost all live foliage were removed for soil baiting and direct root isolation of *Phytophthora cinnamomi*. On completion of the experiment all remaining treated plants had their roots washed free of soil, were inspected for lesions and sampled for *P. cinnamomi*. Samples were taken from any lesions and from the fine feeder roots and collar of the plant. The root samples were surface sterilised and direct plated for the presence of *P. cinnamomi* on P10VP agar. The roots of control plants were also washed free of soil and inspected for root damage if they died during the study. Soil from all surviving treated and control plants was subsampled, including root material, and baited for *P. cinnamomi* at the completion of the trial.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No leaf yellowing 0%</td>
</tr>
<tr>
<td>B</td>
<td>leaf yellowing &lt; 10%</td>
</tr>
<tr>
<td>C</td>
<td>leaf yellowing 10–20%</td>
</tr>
<tr>
<td>D</td>
<td>leaf yellowing 20–50% often some leaf loss</td>
</tr>
<tr>
<td>E</td>
<td>leaf yellowing 50–80% and leaf loss</td>
</tr>
<tr>
<td>F</td>
<td>leaf yellowing &gt; 80% and leaf loss</td>
</tr>
</tbody>
</table>

*Phytophthora cinnamomi* isolations were obtained by flooding the soil/root samples in beakers with distilled water, baiting with lupin radicals for 2–3 days, and culturing the lupin roots on P10VP agar for a week at room temperature (Ribeiro 1978). Colony shape and mycelial morphology were used to identify *P. cinnamomi*. Root isolations were conducted by the surface sterilisation of roots in a 3% NaOCl solution for 1 minute, or 30 seconds for fine feeder roots, and plating on P10VP agar for a week at room temperature (Ribeiro 1978).

**RESULTS**

The results of the study are summarised in Table 3, with full health and isolation data presented in Appendix 3 and images of the treated and control plants at the completion of the trial in Appendices 4 and 5, respectively. Prior to the commencement of inoculation with *Phytophthora cinnamomi* symptoms of disease were observed in some plants. One *Boronia gunnii* in the treatment block had yellowing in its leaf tips (Plate 2), while a *Boronia hemichiton* in the control block had leaf yellowing and curling of the leaf tips (Plate 3). Several of the larger *Philotheca freyciana* plants suffered wholesale leaf loss in June 2007 (Plate 4), a presumed consequence of waterlogging due to inadequate drainage — all plants had regained foliage by August 2007 after the drainage issue was addressed. None of the symptoms were typical of *P. cinnamomi* and no *P. cinnamomi* was isolated from drainage water from any plants in the pre-inoculation baiting. Plant condition stabilised prior to the commencement of inoculation in November 2007.

All the *Aotus ericoides* plants were dead within nine weeks of the first inoculation. The treated *Leptospermum scoparium* plants generally stayed in good health for the duration of the trial with the exception of one death; *Phytophthora cinnamomi* was isolated from the plant’s roots, and there had been some leaf loss, but there was no chlorosis observed.

*Philotheca freyciana* was notable in that the health of the treated plants declined substantially during about 8 weeks from the first inoculation, with one death (*no Phytophthora cinnamomi* was isolated from its roots). The remaining plants stabilised and persisted to the end of the study, although their health was quite poor (see Appendix 4); silver leaf discoloration was apparent on most of the plants, in some instances being confined to a single branch (Plate 5). The control plants did not lose health to the same extent.

The treated *Boronia gunnii* plants were stable for the duration of the study. One treated *Boronia hemichiton* plant remained in good health, while the other two died (one returning a positive root isolation for *Phytophthora cinnamomi*). Three of the treated *Boronia hippocala* plants remained in good health for the duration of the study, while one suffered some minor yellowing of its leaves.
The results of the root isolations undertaken for all surviving treated plants are presented in Appendix 6. No *Phytophthora cinnamomi* was detected in the combined soil and root isolations undertaken for each surviving control plant at the end of the study.

**Table 3.** Plant mortality and *Phytophthora cinnamomi* isolations at completion of the trial.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treated plants.</th>
<th>Total treated Plants.</th>
<th>Surviving treated plants.</th>
<th>Control plants.</th>
<th>Dead control plants.</th>
<th>Surviving control plants.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Live/dead</td>
<td>Number of positive root isolations</td>
<td>Number of positive soil isolations</td>
<td>Live/dead</td>
<td>Number of positive root isolations</td>
<td>Number of positive soil isolations</td>
</tr>
<tr>
<td><em>Boronia gunnii</em></td>
<td>5 / 0</td>
<td>1</td>
<td>2 and 1*</td>
<td>2 / 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>B. hemichiton</em></td>
<td>1 / 2</td>
<td>1</td>
<td>1</td>
<td>1 / 1</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td><em>B. hippocala</em></td>
<td>4 / 0</td>
<td>0</td>
<td>3</td>
<td>2 / 0</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td><em>Philotheca freyciana</em></td>
<td>6 / 2</td>
<td>0</td>
<td>5 and 1*</td>
<td>4 / 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Aotus ericoidea</em></td>
<td>0 / 11</td>
<td>11</td>
<td>N/A</td>
<td>6 / 0</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td><em>Leptospermum scoparium</em></td>
<td>10 / 1</td>
<td>1</td>
<td>5 and 2*</td>
<td>6 / 0</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

* Plate swamped by rapid growing *Pythium*-like mycelium potentially affecting the identification of *Phytophthora cinnamomi*.

**Plate 2.** *Boronia gunnii* with colouring of leaf tips prior to inoculation.

(G6, 25 May 2007)

**Plate 3.** *Boronia hemichiton* with leaf yellowing and curling of tips prior to inoculation.

(H1, 25 May 2007)
DISCUSSION

The susceptibility of plants to Phytophthora cinnamomi is a function of the pathogen, environment and host plant. In undertaking a susceptibility trial in a glasshouse it is recognised that the environmental conditions are substantially different to those experienced by the natural populations of the species tested which may lead to different plant reactions to infection (Barker & Wardlaw 1995; Shearer & Dillon 1996). A natural demonstration of this is the differing susceptibility observed for some species between different biomes or soil types in the field (Shearer & Dillon 1996).

Phytophthora cinnamomi is considered to be a pathogen with little variability in pathogenicity between isolates (Podger 1989). The use of fresh isolate of the pathogen collected from the region of the plant populations tested is considered to be an adequate representation of the pathogenicity of P. cinnamomi that the natural populations may be exposed to.

Species that host Phytophthora cinnamomi may range in response from 100% mortality to field resistance. Consequently, where only one or a limited number of genotypes are tested the interpretation of the results at the population level is problematic.

The numbers of plants and diversity of parent sources of cuttings available to this study was appreciably reduced by the time the inoculations were feasible, severely limiting the interpretation of the susceptibility of the target species to Phytophthora cinnamomi. An additional problem encountered was the regulation of the temperature in the glasshouse and the interaction with the watering regime which put periodic stress on the plants causing some leaf loss, particularly for those plants in small tubes.

However, the glasshouse conditions of flood inoculation, susceptible soil conditions (temperature, moisture and medium) are highly conducive to disease expression, and the results in such conditions may therefore reflect the greatest potential field susceptibility. The results of the treatment of the known susceptible Aotus ericoides plants, in which all plants that were treated died and Phytophthora cinnamomi was recovered from the roots, indicated that the method was successful in introducing infective inoculum to the plants. At no time was P. cinnamomi isolated from drainage water or soil root samples taken from the control plants. The highly susceptible Aotus ericoides plants in the control remained healthy for the six-month duration of the study.

The Leptospermum scoparium resistant control provided an unexpected mortality in the treated plants.
from which *Phytophthora cinnamomi* was isolated from the roots. Otherwise inoculated plants remained essentially healthy except for some leaf loss experienced across both the control and treated plants, a likely result of droughting in the small tubes.

A *Pythium*-like 'lower fungi' was found to be common across many of the isolations taken from soil and root material in this study. It was very rapidly growing and reduced the detection capacity for *Phytophthora cinnamomi* in some circumstances.

**Boronia gunnii**

*Boronia gunnii* is a riparian species known from three populations, only two of which are extant (along the upper reaches of the St Pauls and Apsley Rivers). The populations are very small with the largest estimated to be 600 mature plants (Threatened Species Section 2005a). All plants in the treated block remained healthy throughout the study. One plant died in the control block but no *Phytophthora cinnamomi* was isolated from this plant. *Phytophthora cinnamomi* was present in two of the treated plant pots at the completion of the study. An isolate from the third pot was swamped by a *Pythium*-like 'lower fungi': *P. cinnamomi* was isolated from the roots of this plant though the roots themselves remained healthy (Plate 6).

The results indicate that *Boronia gunnii* can host *Phytophthora cinnamomi* but appears to be resistant to disease.

**Boronia hemichiton**

*Boronia hemichiton* is known from the western flanks of Mt Arthur in northern Tasmania where it grows in wet heath communities. It has a linear range of less than 4 km, with a population size of 1000–2000 mature plants (Threatened Species Section 2005b).

During the course of the study, two of the treated *Boronia hemichiton* plants died and one control plant died. *Phytophthora cinnamomi* was isolated from only one dead treated plant. No *P. cinnamomi* was isolated from the dead control plant. It is likely the plants had experienced another stress in addition to *P. cinnamomi*. At the completion of the trial the remaining treated plant was found to be growing in soil infected with *P. cinnamomi* but no *P. cinnamomi* was isolated from its roots (though it appeared that some of its fine feeder roots were unhealthy).

As there was mortality in the control plants, as well as a treated plant from which *Phytophthora cinnamomi* was not isolated, it is possible that *P. cinnamomi* was not the primary cause of death. Nevertheless, *Boronia hemichiton* has been demonstrated to be a host for *P. cinnamomi* and, until further evidence is available, should be considered to be at least slightly susceptible to the disease.

**Boronia hippopala**

*Boronia hippopala* grows at mid altitudes in Tasmania's Eastern Tiers with an estimated population size of less than 10,000 mature plants (Threatened Species Section 2005c).

The plants in both the control and treatment blocks remained healthy throughout the trial. *Phytophthora cinnamomi* was not isolated from the roots of the treated plants, though it was present in the pots of treated plants at the end of the trial. Some fine feeder root damage was observed on one plant at the completion of the trial. These results suggest that *Boronia hippopala* will not host *P. cinnamomi*.

**Philotheca freyciana**

*Philotheca freyciana* has a highly restricted distribution in the Hazards area of Freycinet National Park, with less than 100 plants known (Threatened Species Section 2006). The plants treated in this study came from eight different wild plants clustered around a granite outcrop to the east of Mt Mayson, and it is likely that the results would be representative of the response of the population to *Phytophthora cinnamomi*.  

No control plants died during the course of the study. Two treated plants died during the study but no *Phytophthora cinnamomi* was recovered from the roots of either plant. Plants in both the control and

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1 It should be noted, however, that the possibility exists that the tested plants may be the progeny of one plant.
Phytophthora cinnamomi susceptibility trials 2007–2008

treatment blocks had experienced branch dieback and patchy chlorosis of the leaves prior to and during the trial. Stresses in addition to *P. cinnamomi* were clearly evident on the plants. Soil and root isolations conducted following completion of the trial resulted in isolation for *P. cinnamomi* from all but one of the treated plant pots. However, *P. cinnamomi* was only isolated from the roots of one plant. The washed roots of the treated plants appeared healthy, with no obvious lesion development (Plate 7).

The results suggest that *Philotheca freyciana* has a high level of resistance to infection by *Phytophthora cinnamomi*. However, it appears that a proportion of the population may host the pathogen or that the pathogen may be effectively prevented from spreading within its host. It is considered likely that the wild population of *Philotheca freyciana* will be resistant to *P. cinnamomi*.

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**OVERVIEW**

Comparison of the results of this study with previous records for the susceptibility of Tasmanian *Boronia* species to *Phytophthora cinnamomi* indicates a greater variability in susceptibility than reported in Podger *et al.* (1990a). *Boronia* plants inoculated in this study displayed a high level of resistance, unlike the field-based study by Podger (1989) that recorded a high level of susceptibility for a combined grouping of *Boronia pilosa* and *Boronia parviflora* in buttongrass moorland vegetation (both species sensu Buchanan 1999). *Boronia citriodora* (sensu Buchanan 1999) has been observed by the authors to be highly susceptible to *P. cinnamomi* in buttongrass moorland in southwestern Tasmania, whereas healthy plants of the Freycinet endemic *Boronia rozefeldsii* have been observed within *Phytophthora cinnamomi* infestations on Schouten Island, indicating field resistance for this species. Nationally thirteen *Boronia* species have been reported as being potential hosts of *P. cinnamomi* (O’Gara *et al.* 2005), with the species’ rating for susceptibility varying from field resistant to highly susceptible. Such intra-genera variability in susceptibility has been recorded for other genera, e.g., *Epacris* in Tasmania (Barker & Wardlaw 1995), or for a range of genera (Shearer *et al.* 2004).

Intra-specific variation in susceptibility has been reported from glasshouse studies (Shearer *et al.* 2004).
Phytophthora cinnamomi susceptibility trials 2007–2008

2004) and the field (Podger 1989; Wills 1992). This attribute has been exploited in the breeding of Phytophthora cinnamomi resistance in some taxa, e.g., Eucalyptus marginata (Shearer & Tippett 1989). The present study displayed little variation in intra-specific variation within the threatened species tested, though it should be borne in mind that the numbers of genotypes inoculated was very low.

In the absence of coincidence of Phytophthora cinnamomi and the threatened species in the field, glasshouse test results must be used as the basis for risk assessment. However, the caveat is that there is some risk of field susceptibility that may differ from that of young plants in glasshouse conditions (Table 4).

Table 4. Response of the target species to Phytophthora cinnamomi.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host to P. cinnamomi?</th>
<th>Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boronia gunnii</td>
<td>yes</td>
<td>resistant</td>
</tr>
<tr>
<td>Boronia hemichiton</td>
<td>yes</td>
<td>slightly susceptible</td>
</tr>
<tr>
<td>Boronia hippopala</td>
<td>no</td>
<td>–</td>
</tr>
<tr>
<td>Philotheca freyciana</td>
<td>yes</td>
<td>resistant</td>
</tr>
</tbody>
</table>

REFERENCES


Threatened Species Section (2006). Listing Statement for *Philotheca freyciana* (Freycinet waxflower). Department of Primary Industries and Water, Tasmania.