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Susceptibility of Selected Tasmanian Rare Plants to *Phytophthora cinnamomi*

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Abstract

Forty-seven species native to Tasmania were tested for their susceptibility to *Phytophthora cinnamomi*. Plants were propagated from cuttings and or seed. The plants were root inoculated by irrigating the moist growing medium with infected broth. Individuals in 36 species proved to be susceptible, four species were resistant hosts, three species resistant and four species returned inconclusive results. *Epacris* contained the most suceptible species and the level of susceptibility varied considerably within the *Epacris*, but also in *Acacia* and *Pultenaea*. Species from a number of genera contained an apparently resistant element.

Introduction

Phytophthora cinnamomi is a soil and root inhabiting, microscopic fungus. In Tasmania, it can cause serious disease in heaths, dry sclerophyll forests, moorlands and disturbed rainforests growing below 600 m and receiving more than 600 mm rainfall annually (Podger *et al.* 1990*b*). There are relatively few large areas free of disease. Podger *et al.* (1990*b*) have demonstrated that 136 Tasmanian native plants are hosts to *P. cinnamomi*. Many of these species have proved to be susceptible to disease and are from a range of families, with the Epacridaceae, Fabaceae, Myrtaceae, Proteaceae and Dilleniaceae, in particular, being affected. Of the known susceptible genera in the Tasmanian flora, most are rare or threatened species. It is this portion of the flora that is the subject of the present research. In the absence of a significant resistant element in a species, there is a real risk of extinction and *ex situ* conservation may be necessary.

Within large infected areas there is a mosaic of infected and uninfected vegetation. The mosaic provides opportunities to manage small areas that are free of infection if they are offered a degree of topographic protection. Topographic protection refers to landscape features, such as ridges and gorges, that can slow or stop the natural spread of the fungus. Where rare plant populations are isolated in suitable areas there is potential for successful protection of this important portion of biological diversity.

The aim of this research was to identify the portion of Tasmania's rare higher plant species that are susceptible to *P. cinnamomi* so that protective management can be implemented. This paper reports the results of susceptibility tests on 47 species that are rare in Tasmania. Related research identifies priority management areas for protection from infection by *P. cinnamomi*.

Materials and Methods

Fifty-six rare or threatened Australian plants (ROTAP) (Briggs and Leigh 1988) (Table 1) were chosen because of their close taxonomic affinities with known susceptible genera and occurrence within the area climatically suitable for the expression of disease caused by *P.cinnamomi*. Of these species, we failed to propagate five species; two were excluded due to taxonomic uncertainty in Tasmania and two

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species could not be relocated. Voucher specimens of each species are located at the Tasmanian Herbarium. Distributional data were collected from herbaria, personal records and published literature. Plants were obtained from two sources: (1) cuttings and seed; and (2) cores containing seedlings from the field.

Table 1. Definitions of the geography and conservation status of rarity in Tasmania after Kirkpatrick *et al.* (1991)

Code	Definition
r1	Rare in Tasmania, restricted to an area < 100 km × 100 km
R1	Nationally rare, restricted to an area < 100 km × 100 km
r2	Rare in Tasmania, restricted to < 20 100 km^2 map cells
R2	Nationally rare, restricted to < 20 100 km^2 map cells
r3	Rare in Tasmania, small localised populations not r1 or r2
R3	Nationally rare, small localised populations not R1 or R2
v	Vulnerable in Tasmania
V	Nationally vulnerable
e	Endangered in Tasmania
E	Nationally Endangered

Propagation

Cuttings or seeds were collected from one population of each species. Cuttings were removed from 25 different individuals of varying vigour that were scattered throughout the population. Seeds were collected from a similar range of plants. All plants were labelled so that individual genotypes could be recognised. Cuttings were struck using standard horticultural techniques under spray on a heated bed. Successful strikes were transplanted into slimline pots filled with potting mix (70% organic matter, 30% sand, pH 6.5) and fertilised with Osmocote. All plants were kept in a glasshouse until new growth was advanced and the root space was occupied, i.e. the plants were actively growing and not root bound.

Wild Seedlings

Soil cores containing wild seedlings were removed where seedlings existed. This was done for five species. For each species, three cores where removed using a spade. Each core measured 35 cm × 40 cm and was trimmed to a depth of 10 cm, placed in a tray in a glasshouse and watered regularly until new growth was seen. No fertiliser was added.

Inoculation

The *P. cinnamomi* used to inoculate the plants was isolated from *Pultenaea hibbertioides* near Pipers River (500700 448500 Tasmap 8215). Fifteen plants (or as many as available if less than 15) of different genotypes for each species were inoculated and five uninoculated additional genotypes were reserved as controls. Two cores of field soil with seedlings were inoculated and one was reserved as a control. The inoculum was grown in 'V8' juice broth prepared according to the method of Ribeiro (1978). *Phytophthora cinnamomi* was grown in a clarified and autoclaved 'V8' vegetable juice medium. After about four days incubation at 20°C on a shaker, the *P. cinnamomi* was macerated using a blender. The inoculum was introduced at a rate of 10 mL per pot by irrigation of the moist growing medium. The process was repeated after 14 days to ensure infection. The glasshouse was maintained between 15°C and 30°C for the duration of the experiment.

A plant was removed from the experiment if death occurred, otherwise symptoms were recorded at the end of the experiment after 120 days. The time in days since inoculation was recorded at the death of each plant. Isolation of *P. cinnamomi* from root tips of all dead plants was attempted. At the end of

the experiment, isolation of *P. cinnamomi* from the remaining plants and from the medium in which they grew was also attempted. The roots of each plant were washed and soaked in 0.5% sodium hypochlorite solution for 30 min. Root tips, collars and obvious areas of infection were removed and placed on $P_{10}VP$ selective agar and incubated for 48 h at room temperature. The presence or absence of *P. cinnamomi* was determined by observation (under a microscope) of the fungus growing on the agar. For any plant from which *P. cinnamomi* could not be isolated, the growing medium was tested for the fungus using the lupin bait technique (Cho 1983).

Susceptibility Assessment

For each species, the number of live individuals and the number of symptomatic individuals were counted. Recovery of *P. cinnamomii* from each individual was recorded as + or –, and totals for live and dead plants were recorded. The total period over which death occurred was recorded.

Each species was rated according to susceptibility following a rating method similar to that of Podger and Brown (1989). The ratings were:

(*a*) resistant (R) species in which individuals showed no symptoms and from which *P. cinnamomi* was not isolated;

(*b*) resistant host (RH) species that showed no symptoms of infection and from which *P. cinnamomi* was isolated;

(*c*) slightly susceptible (SS) species in which the majority of individuals were symptom-free but vigour was depressed in all plants;

(*d*) moderately susceptible (MS) species in which there was considerable mortality (between 25% and 75%) but in which some plants survived and grew despite an apparent depression of vigour; and

(*e*) highly susceptible (HS) species in which there appeared to be little or no resistant element in the populations and in which > 75% were eliminated within the period of the trial (4 months).

Results

The 45 species tested showed varied responses to inoculation with *P. cinnamomi* ranging from complete resistance to high susceptibility (Table 2). The interval of time after inoculation, over which species became diseased and died, varied from less than 40 days for *Acacia axillaris* and *Allocasuarina duncanii* to greater than 110 days for a number of *Epacris* spp., *Hovea corrickiea* and *Bossiaea obcordata.*

Within the two families that were well represented in the trial, susceptibility varied from resistant to highly susceptible among the Fabaceae, and from slightly susceptible to highly susceptible among the Epacridaceae. Congeneric variation existed in *Epacris* and *Pultenaea* in particular. The slight susceptibility of *E. acuminata* contrasts to that shown in the other *Epacris* species, and the resistance of *P. selaginoides* is in contrast to that shown in the other *Pultenaea* species. Congeneric differences between species exist in other genera, for example, *Allocasuarina duncanii* (HS) contrasts to its congeneric *A. crassa* (MS) in which mortality was spread over 120 days and which had some resistance in the sample. Other noteworthy results are those for *Hakea ulicina* (SS) and *Milligania johnstonii* (R).

Among species that showed considerable or complete resistance, some individuals also returned negative soil isolations as well as negative root isolations.

It is not certain whether or not *Stylidium despectum, S. perpusillum* and *Mitrasacme distylis* are susceptible, due to difficulty in isolating *P. cinnamomi* from the very fine and small roots of these species. The surface sterilisation procedure may have killed the fungus inside the very fine roots.

Seedlings in native soil suffered a similar mortality rate to their counterparts in fertilised potting mixture (Table 3). The relatively low numbers of plants in each sample would make comparisons of susceptibility ratings dubious. However, it took a marginally longer time for *Pultenaea hibbertioides* and *Epacris myrtifolia* to die in the native soils. All of the non-target species that died in the field soils were previously known to be susceptible to *P. cinnamomi.*

The depression of vigour was a symptom used to class species as slightly susceptible. Table 4 summarises the growth in height of the slightly susceptible species. The effect on

Table 2. Species responses to inoculation with *P. cinnamomi*

cons; conservation status as described in Table 1. (s), grown from seed (otherwise cuttings); cons = conservation status as defined in Table 1; sus = susceptibility rating as defined in the methods; *n* = number of plants inoculated; dead = number of plants that died during the experiment; pos = *P. cinnamomi* isolated from roots; live = number of plants remaining alive after the experiment; soil+, number of negative remaining live plants that returned positive soil; time = period over which deaths occurred in days. Superscripts; * *P. cinnamomi* has been isolated as the cause of field mortality; ** has been recorded in *P. cinnamomi* infected vegetation with no apparent symptoms; + have been observed dead in the field in *P. cinnamomi* infected soil but the pathogen has not been isolated from the species; ++ species so small *P. cinnamomi* may have been killed during root surface sterilisation before isolation was attempted

Species	cons	sus	*n*	dead	pos.	live	pos.	soil +	time
Acacia axillaris (s)	Rr2u	MS	15	4	4	11	2	4	31
A. pataczekii	Rr2u	SS	15	–	–	15	15	–	–
A. siculiformis (s)	r2	MS	15	8	8	7	1	5	71
Allocasuarina crassa (s)	Rr1	MS	15	6	6	9	7	2	120
A. sp. nov. (s)	Rr1	HS	8	8	8	–	–	–	38
Acrotriche cordata	r1	HS	6	5	5	1	1	–	54
Bossiaea obcordata	r1u	SS	5	2	1	3	1	1	113
Callistemon pallidus	r3	RH	15	–	–	15	6	3	–
Cyathodes pendulosa	Rr2	HS	4	2	2	2	2	–	102
Dianella longifolia (s)	r2	HS	2	1	1	1	–	–	24
Epacris acuminata	Rr2	SS	15	2	1	12	11	1	74
E. apsleyensis	Rr1	HS	3	3	2	–	–	–	81
*E. barbata**	V	MS	6	3	3	3	–	–	85
*E. curtisiae**	u	HS	15	15	15	–	–	–	75
E. exserta	Rr3	MS	15	10	10	5	5	–	120
E. glabella	Rr1u	MS	8*	4	4	–	–	–	88
E. grandis	Rr1	HS	4	4	4	–	–	–	112
*E. limbata**	V	HS	4	4	4	–	–	–	75
E. marginata	Rr1	HS	7	7	7	–	–	–	79
E. myrtifolia	Rr2	HS	15	14	14	1	1	–	113
E. paludosa	r2	HS	4	3	3	1	1	–	109
E. stuartii	V	MS	5	4	3	–	–	–	109
E. virgata	V	HS	6	5	5	1	–	–	85
Hakea ulicina (s)	r2	SS	15	4	–	10	5	–	–
Hibbertia calycina	vu	HS	2	2	2	–	–	–	81
H. virgata	r2	HS	2	2	2	–	–	–	54
Hovea corrickiae (s)	r1	MS	14	6	5	8	8	–	111
Helicrysum lycopodioides	Rr1	RH	15	–	–	15	3	6	–
*Leucopogon esquamatus***	r1	MS	3	3	2	1	–	–	107
L. lanceolatus	r2u	HS	10	9	9	1	1	–	75
Milligania johnstonii	Rr1	R	70	–	–	70	–	–	–
Mitrasacme distylis++*	r1	?	34	21	1	13		13	41
*Melaleuca pustulata***	Rr1	RH	15	–	–	15	12	3	–
Persoonia meulleri var. *densifolia*	Rr1	HS	2	2	2	–	–	–	106
Phebalium daviesii	Eu	HS	5	5	5	–	–	–	66
Platylobium formosum var. *floribunda*	r2	SS	3	–	–	3	3	–	–

Table 2 *continued*

Species	cons	sus	*n*	dead	pos.	live	pos.	soil +	time
Prostanthera rotundifolia	vu	RH	15	–	–	15	4	6	–
*Pultenaea hibbertioides**	vu	HS	12	12	11	–	–	–	53
P. paleacea	v	HS	15	13	12	2	1	1	65
P. prostrata	vu	HS	15	15	15	–	–	–	81
P. selaginoides (s)	V	R	15	–	–	15	–	9	–
Richea dracophylla (s)	Rr2	HS	15	12	12	3	–	1	93
Sprengelia distichophylla	Rr2	R	3	–	–	3	–	–	–
Stylidium despectum++	r2	?	7	7	–	–	–	–	–
S. perpusillum++	r2	?	25	11	–	14	–	14	–
Xanthorrhoea bracteata+	r2	?	–	–	–	–	–	–	–
*X. arenaria**	r2	HS	–	–	–	–	–	–	–

growth in height varies. For *Epacris acuminata* and *Hakea ulicina,* growth in height is limited to half that of the untreated sample. The result from the resistant *Pultenaea selaginoides*, where the infected sample grew faster than the untreated sample is unexpected. This result may be a reflection of the fertilising effect of the 'V8' broth containing the inoculum because this was not applied as a control (without inoculum) to the untreated sample.

Discussion

This study has established the level of susceptibility of 36 species not previously known to be susceptible to *P. cinnamomi*. *Phytophthora cinnamomi* has been isolated from dead and dying plants of five of the species in the field (Table 2). As all of the species occur in habitats that are conducive to *P. cinnamomi* damage, these results demonstrate that *P. cinnamomi* represents a considerable threat to the conservation of these rare plants in Tasmania.

It is clear from the results of this study that there is considerable variation in the expression of disease within the genera examined. This is particularly evident in *Epacris* and *Pultenaea.* The latter has a resistant species and otherwise highly susceptible species. The level of resistance within species is variable and considerable. It should be noted that the experimental environment was favourable to the growth of *P.* cinnamomi and that the host responses may be different in the field. We expect that the expression of disease in the field would be effected by environmental variation including variation in annual rainfall and temperature. The effect of drought is discussed later.

Although we are hesitant to derive too much ecological significance from the results of this glasshouse trial it is reasonable to suggest that the apparent susceptibility and more importantly, resistance, is real and can be used as a guide to the level of threat to these species in the field. However, it has been demonstrated that there are differences in susceptibility among provenances of *Eucalyptus regnans* (Harris *et al.* 1985) and so variation in susceptibility may be expected between different populations of the species tested in the present study. The differences in susceptibility within families of provenances of *E. regnans* suggest that any material from the present suite of species to be considered for *ex situ* conservation or reintroduction should be bred with this potential in mind. Clonal material derived from resistant stock would be desirable.

Table 3. Species lists and the number of deaths and survivors recorded in a control and in two inoculated trays containing wildlings removed from the field in native soil (Reps 1 and 2)

The selected rare species are given in bold

Species	Control Deaths	Rep. 1 Deaths	Rep. 2 Deaths	Time
Pultenaea hibbertioides	0/3	3/3	1/2	75
Epacris impressa	0/4	2/3	4/5	
Leucopogon collinus	0/1	2/3	–	
Goodenia lanata	0/2	0/4	0/2	
Gahnia grandis	0/4	0/5	0/4	
Allocasuarina littoralis	0/1	–	0/1	
Asplenium flabellifolium	0/2	0/4	–	
Bossiaea obcordata	0/2	0/2	–	
Pultenaea gunnii	0/1	1/1	–	
Gonocarpus tetragynus	0/3	0/2	–	
Epacris impressa	0/1	1/1	–	
Epacris curtisiae	0/10	8/8	7/7	100
Boronia citriodora	0/10	4/12	–	
Sprengelia incarnata	0/14	13/17	21/21	
Epacris obtusifolia	0/6	1/7	0/7	
Baeckea leptocaulis	0/4	2/5	3/10	
Schoenus tenuissimus	0/12	–	0/20	
Baeura rubioides	0/10	0/10	0/23	
Leptospermum lanigerum	0/8	–	0/1	
Leptocarpus tenax	0/18	0/16	0/16	
Empodisma minus	0/8	0/10	0/6	
Acacia myrtifolia	0/3	2/3	1/1	110
Helichrysum sp.	0/2	0/2	0/1	
Leptospermum scoparium	0/1	0/1	–	
Milligania johnstonii	0/40	0/33	0/37	–

For those species that had some plants survive the treatment, a number returned negative root isolations and negative soil isolations. This result is most pronounced among those species with considerable resistance and a significant number of plants that did not host the pathogen. In *P. selaginoides,* for example, no plant returned a positive root isolation and 9 of 15 returned negative soil isolations. Similarly, no susceptible plants of *Prostanthera rotundifolia* were found, and 6 of the 11 negative plants returned negative soil isolations. These results contrast to those shown in a typical highly susceptible species, such as *Epacris curtisiae,* in which all 15 plants returned positive root isolations indicating all 15 soils also supported *P. cinnamomi.* This relatively high level of apparent failed inoculations is most strongly associated with the resistant species. The negative soil isolations may therefore indicate *P. cinnamomi* did not persist in the soil due to lack of host material.

Cho (1983) demonstrated that seedlings and cuttings of *Banksia* spp. are not differentially susceptible, so the differences in propagation techniques in the present study (Table 2) should not affect the results. Intra-generic variation in host susceptibility has been suspected in the field and has been demonstrated among *Banksia* species experimentally (McCredie *et*

Table 4. Slightly susceptible species heights as means (and se) in response to the inoculation treatment (T) and control (C)

All species show significantly different growth rates between T and C ($P < 0.05$). * indicates that the treatment mean is significantly higher than the control mean

Species	T mean (se)	C mean (se)
Acacia pataczekii	34 (1.1)	50 (3.8)
Platylobium formosum	21 (2.6)	33 (1.8)
Callistemon palludosus	27 (0.9)	34 (0.4)
Epacris acuminata	9 (0.7)	22 (1.5)
Hakea ulicina	8 (0.5)	15 (0.4)
Hovea corrickiae	39 (2.5)	67 (8.0)
Pultenaea selaginoides	78 (4.3)*	61 (3.4)

al. 1985). Intra-specific resistance has been demonstrated using clones of the susceptible *Eucalyptus marginata* (McComb *et al.* 1987). After inoculating healthy trees, Rockel (1977) presented data (but made no comment) that clearly indicate that remaining and apparently healthy trees of *Eucalyptus marginata* and *Banksia grandis* in diseased forest are in fact the resistant component. The range of results in the present study, from highly susceptible species to a large degree of resistance in populations is supported by these past studies and field observations (Weste 1981).

The reduced growth of slightly susceptible species (Table 4) may be due to the loss of fine roots caused by disease. Fine root loss makes species susceptible to drought in natural habitats (Weste 1975). Slightly susceptible species such as *Epacris acuminata* and *Acacia pataczekii* (Table 2) may succumb to disease in the field where infection is maintained from year to year resulting in slow attrition of populations or collapse during drought. Growth rate is also important in the competitive selection of component species in communities (Grubb 1977). Reduction of growth rate in *Eucalyptus marginata* surviving on infected sites has been demonstrated by Podger (1972) and Crombie and Tippet (1990). Hence, understorey species suffering reduced growth due to infection with *P. cinnamomi* (Table 2) may be suppressed by other species and excluded from otherwise undisturbed communities. Those species that showed very low levels of resistance in the sample, for example, *Pultenaea paleacea* and *Epacris myrtifolia* may also be placed at a competitive disadvantage in natural regeneration.

The shift in dominance to resistant species, particularly monocots, has been demonstrated in previously shrubby sclerophyll woodlands (Weste 1981). The demise of the present species would also greatly reduce the integrity of the communities that currently support them. Genetic depletion may pose a longer term threat to these species. Obviously this is all the more significant where the excluded species are rare or threatened.

Generally, the susceptible species are representatives of heathland and heathy forest and woodland understoreys. *Pultenaea prostrata* is from grassy woodland and *Hovea corrickiae* and *Acacia pataczekii* are from moister forest types. All of the tested species are an integral part of their respective communities which often occur in unusual habitats. Although most of the communities that support these species are not infected with *P. cinnamomi*, the passage of time is likely to see the pathogen continue to spread at current rates unless strategies to ameliorate this are implemented. Where the species are rare and confined to specific habitats any degree of susceptibility may have dire consequences for the conservation of the species. The dynamics of the relationship between the pathogen and the species populations needs to be determined.

The information obtained from the present research can be used to prioritise the species in terms of those needing immediate threat abatement strategies (i.e. those that are highly susceptible) such as germplasm collection, population establishment or specific management prescriptions implemented. Following this research, priority management areas have been identified and specific management strategies have been dedicated to each with the aim of minimising the risk of introducing *P. cinnamomi* to this important component of Tasmania's biodiversity.

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