Susceptibility to *Phytophthora cinnamomi* and Phosphite Effectiveness in the Genus *Lambertia*

An interim report

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Introduction

Lambertia species are mainly woody shrubs, with some species reaching small tree size (Table 1). Fifteen species are currently recognised in Western Australia (Western Australia Herbarium 1998-) and one in New South Wales (Hnatiuk 1995) (Table 1). Five *Lambertia* species are Declared Rare Flora and two species have a P (priority) conservation code (Table 1).

Although *P. cinnamomi* is a major threatening process for a number of *Lambertia* species, the susceptibility of *Lambertia* species to the pathogen is poorly understood or documented. Such information is needed to understand differences in survival of flora threatened by *P. cinnamomi* under different situations.

Table 1. Current species in the genus *Lambertia*, rarity and general habit. The first 15 species are ordered by approximate location in Western Australia.
Source: Western Australia Herbarium (1998-) and Hnatiuk (1995). Declared rare and priority flora conservation code: R = Declared Rare Flora; P3 = poorly known taxa and P4 = rare taxa.

Lambertia species	Abbrv	Location	Rare	Habit
multiflora multiflora	LMM	N sandplains		shrub
multiflora darlingensis	LMD	Swan Plain		shrub
echinata occidentalis	LEO	Whicher Rng	R	tall shrub
rariflora rariflora	LRR	Margaret Rv	P4	tree
rariflora lutea	LRL	Walpole	P3	tree
ilicifolia	LIL	Wheatbelt		shrub
inermis drummondii	LID	Lower Sthn		tall shrub
orbifolia Scott Rv	LOS	Scott Rv	R	tall shrub
orbifolia orbifolia	LOO	Narrikup	R	tall shrub
ericifolia	LE	SRNP		tall shrub
fairallii	LFAI	SRNP	R	shrub
inermis inermis	LII	Sth Coast		tall shrub
uniflora	LU	Sth Coast		shrub
echinata citrina	LEC,LEP	Sth Coast		shrub
echinata echinata	LEE	Le Grand NP	R	shrub
formosa	LFORM	NSW		shrub

Currently aerial application of the systemic fungicide phosphite is being used to protect a number of threatened *Lambertia* species from *P. cinnamomi* infestation. Recent National Heritage Trust funded research by CALM has shown that plant species differences is probably the most important factor affecting the effectiveness of low-volume (LV) application of phosphite for the control of *P. cinnamomi* infection (Shearer *et al*. 2003). LV applied phosphite does not protect some species such as *L. inermis* and *Banksia attenuata*, but gives good protection to other species such as *B. coccinea* and *B. grandis* (Fig. 1).

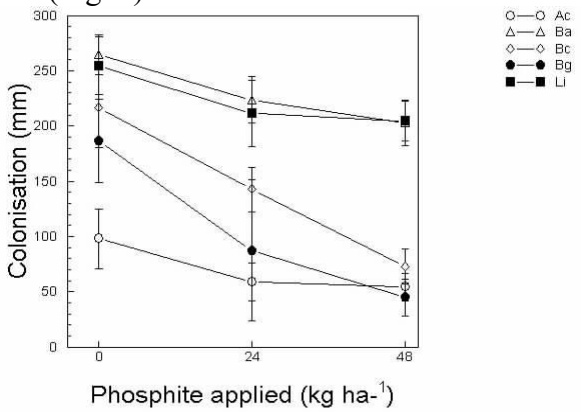

Figure 1 Effect of ultra-low-volume application of phosphite on colonisation of *Phytophthora cinnamomi* in stems of *Adenanthos cuneata* (AC), *Banksia attenuata* (Ba), *B. coccinea* (Bc), *B. grandis* (Bg) and *Lambertia inermis* (Li). Ultra-low-volume applied phosphite is ineffective in protecting *L. inermis* and *B. attenuata* from *P. cinnamomi*.

Reasons for LV applied phosphite differences between plant species is unknown and we know little of which species fall into the ineffective and effective group. The findings have important implications for the management of communities threatened by *P. cinnamomi* using LV applied phosphite. For example LV applied phosphite does not appear to be protecting *L. echinata* ssp *occidentalis* in the ironstone communities (Fig. 2).

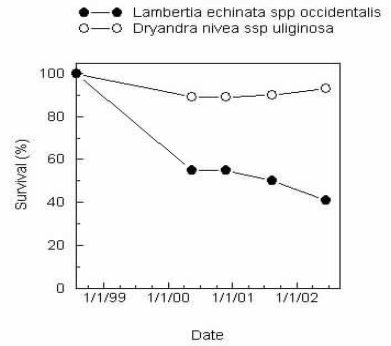

Figure 2 Survival of *L. echinata* ssp *occidentalis* compared to *Dryandra nivea* spp *uliginosa* in an ironstone community infected with *Phytophthora cinnamomi* and ultra-low-volume application of phosphite(Data R. Smith).

Future management of *Lambertia* communities threatened by *P. cinnamomi* requires identification of species for which LV application of phosphite will be either effective or ineffective in the control of *P. cinnamomi*. Once the species in the phosphite effective and ineffective groups have been identified, alternative application strategies can be tested.

In this project all species within the genus *Lambertia* were tested for susceptibility to *P. cinnamomi* and the effectiveness of phosphite against the pathogen.

Materials and Methods

Experimental design

Soil inoculation

Eleven *Lambertia* species were soil inoculated with *P. cinnamomi* in a shadehouse (Table 2). There was insufficient seed to soil inoculate all *Lambertia* species. Pots were blocked by species. *Lambertia* species was the independent variable, time to plant death the dependent variable.

Stem inoculation

All 15 species were stem inoculated with *P. cinnamomi* (Table 2). The independent variables were *Lambertia* species and rate of low-volume applied phosphite (0, 24 and 48 kg/ha). The dependent variables were phosphite levels and lesion development of *P. cinnamomi* in stems. A single plant within a pot was a replicates with 10 replicates per species. Treatments were arranged in a randomised block design in a glasshouse. Inoculated plants receiving no phosphite were used to determine differences in susceptibility between *Lambertia* species to *P. cinnamomi*, in addition to phosphite effectiveness.

Lambertia species

Table 2 gives details of the provenance of *Lambertia* species tested for susceptibility to *P. cinnamomi* and phosphite effectiveness

Table 2. Inoculation type, number of replicates, abbreviation, location and voucher numbers for *Lambertia* species either soil or stem inoculated with *Phytophthora cinnamomi*

Species	Inoculation	Reps	Provenance Abbreviation	Location	Voucher Number
multiflora multiflora	soil,stem	2	LMM1	N sandplains-sand	WAHERB[A]1925520
	soil,stem		LMM4	N sandplains-gravel	
multiflora darlingensis	soil,stem	2	LMDW	Swan Plain-sand	WAHERB6105513
	soil,stem		LMDG	Swan Plain-gravel	WAHERB6253806
echinata occidentalis	soil,stem	2	LEO	Whicher Range	TFSC[B]01694
	soil,stem		LEOms72	Whicher Range	
rariflora rariflora	stem	1	LRR	Margaret River	WAHERB5085594
rariflora lutea	stem	1	LRL	Walpole	WAHERB5426111
ilicifolia	stem	1	LIL	Tarin Rock	TFSC01462
inermis drummondii	soil,stem	1	LID	Stirling Range NP	TFSC01700
orbifolia Scott Rv	soil,stem	2	LOSbe	Scott Rv-Beenup	TFSC01693
	soil,stem		LOSss	Scott Rv-Snake springs	TFSC00303
orbifolia orbifolia	soil,stem	1	LOO	Narrikup	TFSC01696
ericifolia	soil,stem	1	LE	Stirling Range NP	TFSC01698
fairallii	stem	1	LFAI	Stirling Range NP	TFSC00094
inermis inermis	soil,stem	1	LII	Sth Coast	NS[C]21341
uniflora	soil,stem	1	LU	Boulder Hill	TFSC01656
echinata citrina	soil,stem	2	LEC	Cheyene Beach	TFSC01697
	soil,stem		LEP	Cheyene Beach	TFSC00141
echinata echinata	stem	1	LEE	Le Grand NP	TFSC00124
formosa	soil,stem	1	LFORM	NSW	

[A]Western Australian Herbarium
[B]Threatened Flora Seed Centre, Department of Conservation and Land Management
[C]Nindethana Seed

Inoculation
Soil

Number of plants tested for *P. cinnamomi* susceptibility by soil inoculation is given in Table 3. There was insufficient seed of 5 *Lambertia* species for testing of susceptibility to *P. cinnamomi* by soil inoculation (Table 3).

Germinated seeds of *Lambertia* species were transplanted into potting mix of 1 part German Peat to 3 parts river-washed-sand in 15-cm-diameter pots and fertilised once with 3 g of Yates Nutricote® No. 10 controlled release fertiliser (20N:0P:10.8K). A 12-cm-long, 1-cm-diameter screw capped plastic tube was pushed into the soil at the centre of each pot after planting. At the time of soil-inoculation the plastic tubes were removed and *Pinus radiata* inoculum plugs inserted into the holes without damaging roots. The pine inoculum plugs were colonised with each of three isolates of *P. cinnamomi*, as described by Butcher *et al*. (1984). The isolates of *P. cinnamomi* used for soil-inoculation were virulent isolates that represented the geographic range of *P. cinnamomi* in the South-West Botanical Province: DP4 from the Northern Sandplains, DP51 from the Western edge of the wheatbelt and DP55 from the South Coast. Pots containing plants at least 6-months-old were inoculated in mid-summer by removing the plastic tube and inserting one colonised pine plug of each isolate into the hole and covering with soil.

Table 3. Number of plants of *Lambertia* species soil inoculated with *Phytophthora cinnamomi*

Species	Inocul-ation	Reps	Provenance Abbreviation	Number tested
multiflora multiflora	soil,stem	2	LMM1	77
	soil,stem		LMM4	95
multiflora darlingensis	soil,stem	2	LMDW	96
	soil,stem		LMDG	198
echinata occidentalis	soil,stem	2	LEO	223
	soil,stem		LEOms72	123
rariflora rariflora	stem	1	LRR	
rariflora lutea	stem	1	LRL	
ilicifolia	stem	1	LIL	
inermis drummondii	soil,stem	1	LID	145
orbifolia Scott Rv	soil,stem	2	LOSbe	152
	soil,stem		LOSss	217
orbifolia orbifolia	soil,stem	1	LOO	153
ericifolia	soil,stem	1	LE	195
fairallii	stem	1	LFAI	
inermis inermis	soil,stem	1	LII	176
uniflora	soil,stem	1	LU	187
echinata citrina	soil,stem	2	LEC	137
	soil,stem		LEP	192
echinata echinata	stem	1	LEE	
formosa	soil,stem	1	LFORM	94

Stem

Stems were wound-inoculated in November 2004 with *P. cinnamomi* isolate DP55, 5 days after phosphite application using methods previously described (Shearer *et al*. 1987a,b, 1988). Isolate DP55 was chosen because it was a virulent isolate from the Bell Track infection in the Fitzgerald River National Park and used in previous studies (Shearer and Crane 2001, Shearer *et al*. 2003, Shearer *et al*. 2004). An agar disk containing mycelium of DP55 was bound to a fresh cut in the phloem. Control stems were inoculated in a similar manner with sterile agar disks.

Low volume phosphite spray

Low-volume phosphite solution was applied to the foliage using a 'Herbi Microfit' (Micron Sprayers Ltd, Bromyard Herefordshire, UK) low-volume hand-held sprayer (Fig. 2.1). The sprayer delivered a controlled droplet size of 250 μm at 1.78 ml/sec over 1.1 m^2 . Foli-R-Fos 400 (400 g/ L phosphorus acid present as mono-di potassium phosphite adjusted to pH 5.7-6, Unitec Group Pty Ltd) and 0.2% surfactant BS1000 (Cropcare Australasia, Queensland) was sprayed over the foliage for 4 or 8 seconds per plant under still air conditions to give rates of application of 24 or 48 kg/ha, respectively. Rates of 12-24 kg phosphite/ha are currently used to protect rare flora from *P. cinnamomi* infection. Plants receiving no phosphite were sprayed with water and 0.2% surfactant. The surface soil of the pots was covered with tissue to prevent absorption of phosphite into the soil.

Assessment
Soil inoculation

Mortality was recorded thrice weekly for 5 months. Infection was confirmed by plating collar and root pieces onto selective medium (Shearer and Dillon 1995).

Stem inoculation

Fourteen days after inoculation, the bark of harvested stems was carefully scraped from the lesion margins above and below and at each side of the Inoculation point. Visible lesion length above and below the inoculation point and the circumference of lesion and stem diameter at the point of inoculation was measured. Tangential spread at the inoculation point was estimated in degrees.

For determination of colonisation the stems above and below the inoculation point were cut into 0.5-cm-long sections along the lesion and extending 10 cm into apparently healthy tissue. Healthy tissue was cut and plated first with utensils sterilised between cuts and plating. Sections were plated onto half-strength potato-dextrose selective medium of Tsao and Guy (1977) with Rifampicin instead of Pimaricin and Ampicillin instead of Vancomycin. Plated sections were incubated in the dark at 25 °C for 2 days and the number infected with *P. cinnamomi* determined. Colonisation was the length of section infected with *P. cinnamomi*.

Phosphite

Stem samples were washed in distilled water with phosphate-free detergent and dried at 40 °C for several days. An electric grinder with a 1-mm sieved was used to grind dried samples, the grinder being cleaned with compressed air and a fine brush between samples. Ground material was placed in screw-cap containers and sent to the Western Australian State Chemistry Centre where phosphite was determined as the methyl ester by Gas-Liquid Chromatography with flame photometry detection (Spadek, *Western Australian State Chemistry Centre*). Samples were analysed along with two control samples of known phosphite content per 50 samples. Concentrations were confirmed for 10-15% of samples by replicate analysis. The limit of detection was 0.1 μg/g dry weight material (Spadek, *Western Australian State Chemistry Centre*

Temperature

Temperature was recorded with 2 maximum-minimum mercury thermometers in the middle and on each side of the glasshouse. Daily maximum and minimum temperatures were recorded during the inoculation period.

Statistical Analysis

Phosphite effectiveness was analysed by one-way ANOVA for each *Lambertia* species, with phosphite dose the independent variable and colonisation the dependent variable.

Results

Soil inoculation

Time to death was expressed as mortality curves (Fig. 3). The greatest mortality occurred for *L. orbifolia*, *L. echinata* ssp *occidentalis*, *L. ericifolia*, *L. inermis inermis* and *L. uniflora* (Fig. 3). Least mortality occurred for *L. formosa*, followed by the two *L. echinata citrina* and the *L. multiflora darlingensis* from Welshpool. Intermediate between the high and low mortality were *L. multiflora darlingensis* from Gosnels, the two *L. multiflora multiflora* and *L. inermis drumondii* (Fig. 3). Mortality at 100 days (Table 4) followed the same trend as that described for mortality curves.

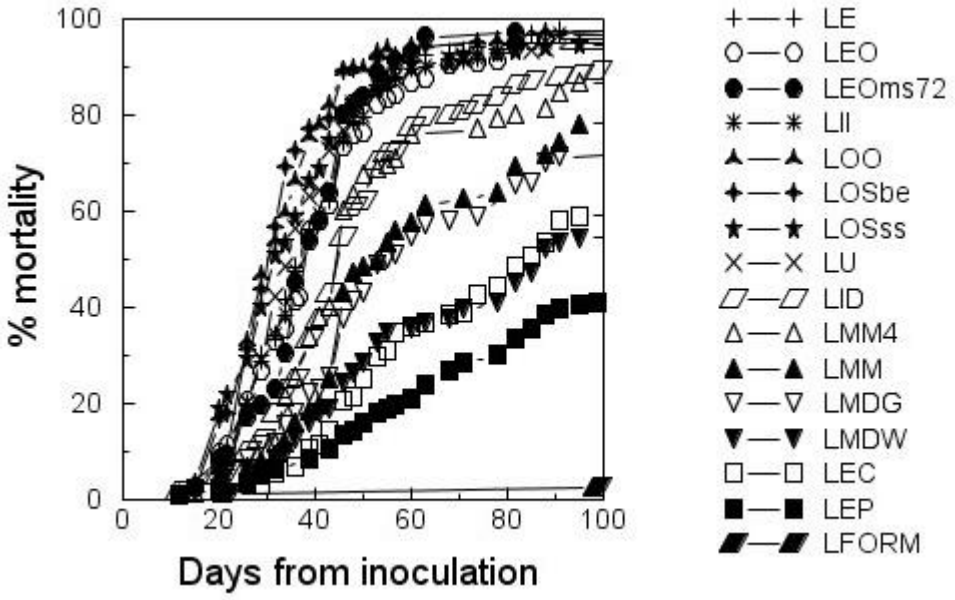

Figure 3 Mortality of 11 species of *Lambertia* following soil inoculation with *Phytophthora cinnamomi* in a shadehouse. See Table 1 for meanings of *Lambertia* name abbreviations.

Stem inoculation

Daily mean maximum and minimum temperature during the inoculation period was 24.4 $\pm$ 0.9 °C and 14.8 $\pm$ 0.5 °C, respectively. There was no significant difference in temperature recorded at the 2 positions within the glasshouse.

Colonisation of *P. cinnamomi* was least for *L. formosa* (Table 4) with the total of 44 mm representing a colonisation extension rate of 0.3 cm/day. Greatest colonisation of the pathogen occurred in *L. orbifolia orbifolia* with an average colonisation extension rate of 1.7 cm/day.

Table 4. Percent mortality of *Lambertia* species 100 days after soil inoculation with *Phytophthora cinnamomi* in a shadehouse and mean stem colonisation (± standard error) for plants not treated with phosphite, 14 days after stem inoculation with *P. cinnamomi* in a glasshouse

Species	Provenance Abbreviation	% Mortality 100 days	Total colonisation (mm)
formosa	LFORM	1	44 ± 8
rariflora rariflora	LRR		48 ± 12
echinata citrina	LEP	41	54 ± 8
echinata citrina	LEC	58	70 ± 8
multiflora darlingensis	LMDG	71	77 ± 15
echinata occidentalis	LEOms72	97	90 ± 16
multiflora multiflora	LMM4	86	95 ± 13
ilicifolia	LIL		96 ± 27
multiflora darlingensis	LMDW	54	98 ± 13
echinata occidentalis	LEO	96	104 ± 13
multiflora multiflora	LMM1	78	108 ± 27
echinata echinata	LEE		113 ± 18
inermis drummondii	LID	89	116 ± 25
inermis inermis	LII	97	126 ± 15
uniflora	LU	94	128 ± 23
orbifolia Scott Rv	LOSss	95	134 ± 19
orbifolia Scott Rv	LOSbe	95	142 ± 24
rariflora lutea	LRL		151 ± 12
fairallii	LFAIR		154 ± 17
ericifolia	LE	97	171 ± 18
orbifolia orbifolia	LOOn	97	233 ± 29

Plotting species mortality after 100 days following soil inoculation against stem colonisation shows the relative susceptibilities of the *Lambertia* species to *P. cinnamomi* (Fig. 4). The most susceptible *Lambertia* species to *P. cinnamomi* were: *L. orbifolia*, *L. echinata* ssp *occidentalis*, *L. ericifolia*, *L. inermis inermis* and *L. uniflora* (Fig. 4). *Lambertia formosa* showed greatest resistance followed by *L. echinata citrina* and the *L. multiflora darlingensis*. Intermediate between the high and low susceptibility to *P. cinnamomi* were *L. multiflora multiflora* and *L. inermis drumondii* (Fig. 4).

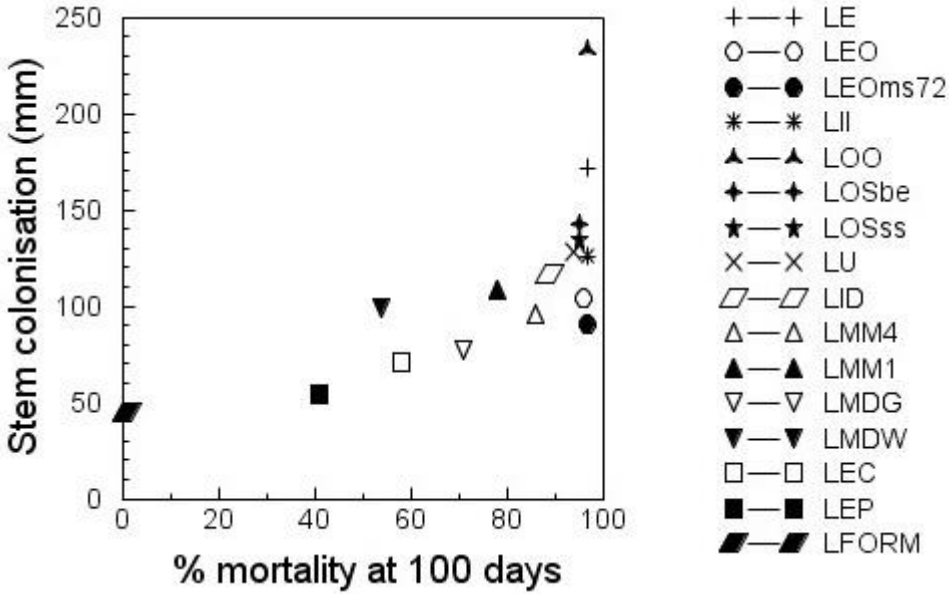

Figure 4 Relationship between percent mortality after 100 days following soil inoculation of *Lambertia* species with *Phytophthora cinnamomi* in a shadehouse and colonisation following stem inoculation of the pathogen into the *Lambertia* in a glasshouse. See Table 1 for meanings of *Lambertia* name abbreviations.

Phosphite analysis

There was considerable variation in phosphite tissue concentrations between *Lambertia* species and between low volume phosphite application (Table 5). High tissue concentrations of over 100 mg phosphite/kg were recorded in *L. formosa*, *L. rariflora rariflora*, *L. inermis drummondii, L. inermis inermis, L ericifolia*, *L. multiflora darlingensis* W, *L. orbifolia orbifolia* and *L. rariflora lutea* (Table 5).

There was a significant increase in tissue phosphite concentration with increased application in only 3 species; *L. formosa,*one provenance of *L. echinata citrina* and one provenance of *L. orbifolia* Scott Rv (Table 5). There was a significant decrease in tissue phosphite concentration with increased application in 2 species; both provenances of *L. multiflora multiflora* and in *L. inermis drummondii* (Table 5). For all the rest there was no significant difference in phosphite tissue concentration between the two application levels.

Table 5. Mean (± standard error) phosphite concentration in stem and leaf tissue of *Lambertia* species following low volume foliar application of 24 and 48 kg of phosphite/ha in a glasshouse
See Table 1 for meanings of *Lambertia* name abbreviations.

Species	Tissue phosphite (mg/kg)	
	after 24 kg phosphite/ha	after 48 kg phosphite/ha
LFORM	166 ± 21	291 ± 64
LRR	129 ± 20	130 ± 22
LMDG	47 ± 10	57 ± 15
LEOms72	59 ± 16	46 ± 21
LMM4	48 ± 6	29 ± 6
LEE	86 ± 22	105 ± 14
LID	177 ± 42	119 ± 15
LII	263 ± 34	244 ± 40
LU	98 ± 12	71 ± 15
LE	132 ± 42	149 ± 41
LEP	45 ± 10	57 ± 21
LEC	31 ± 4	69 ± 16
LIL	-	-
LMDW	152 ± 19	217 ± 63
LEO	56 ± 9	40 ± 5
LMM1	40 ± 4	21 ± 2
LOSss	40 ± 10	86 ± 21
LOSbe	40 ± 7	31 ± 8
LOOn	214 ± 49	172 ± 59
LRL	129 ± 9	122 ± 14
LFAIR	37 ± 5	34 ± 10
BG	33 ± 8	44 ± 14

Phosphite did not significantly affect colonisation of *P. cinnamomi* in 10 *Lambertia* species (Fig. 5). In 8 *Lambertia* species, while phosphite did not significantly affect colonisation of the pathogen trends were apparent; colonisation was less in phosphite sprayed than not sprayed plants (Fig. 5). Phosphite significantly reduced colonisation of *P. cinnamomi* in *B. grandis* and only 2 *Lambertia* species (Fig. 5

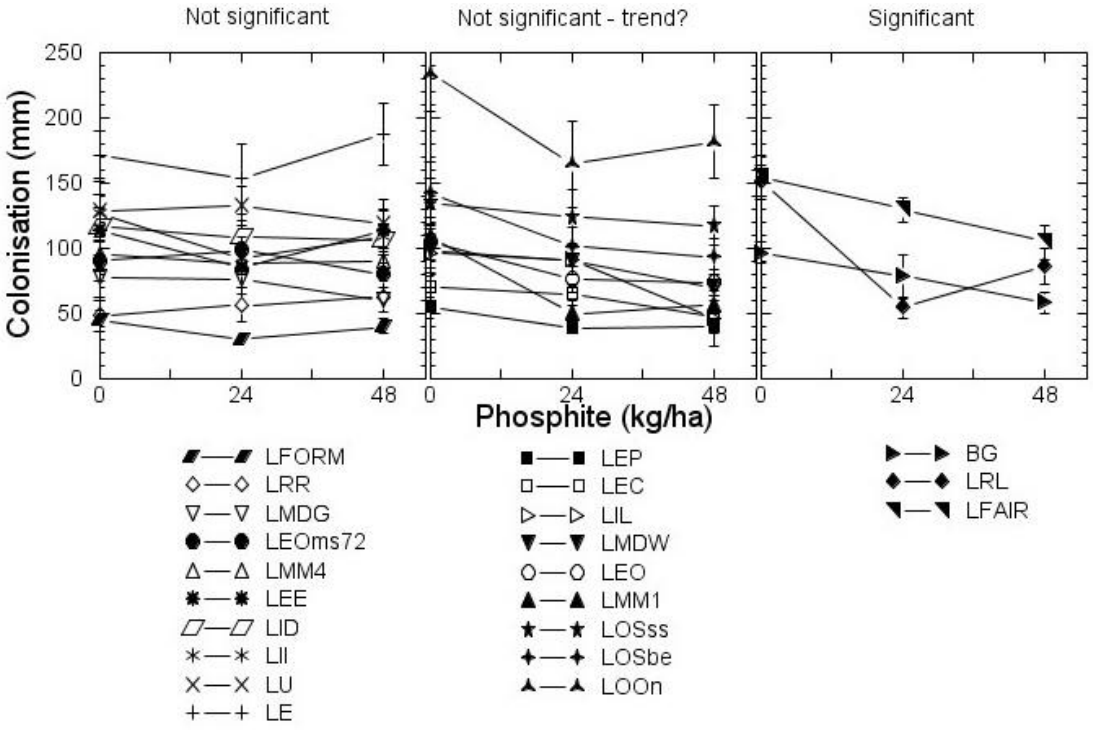

Figure 5 Effect of ultra-low-volume application of phosphite on colonisation of *Phytophthora cinnamomi* in stems of *Lambertia* species for which the interaction with phosphite was either not statistically significant, not significant, but showing a trend or significant. See Table 1 for meanings of *Lambertia* name abbreviations.

Conclusions

Susceptibility

1. Stem inoculation is a more severe test for susceptibility of *Lambertia* spp. to *P. cinnamomi* than soil inoculation.
2. All rare *Lambertia* species are susceptible to *P. cinnamomi*.
3. Four common *Lambertia* species show some resistance to *P. cinnamomi*.
4. Intraspecific variation in resistance to *P. cinnamomi* found within *Lambertia*.
5. The genus *Lambertia* is an ideal model to examine the mechanisms of resistance to *P. cinnamomi*.

Phosphite effectiveness

1. The study confirmed the effectiveness of phosphite in *B. grandis* and ineffectiveness in *L. inermis inermis*.
2. there was no significant control of *P. cinnamomi* in most rare *Lambertia* species.

3. In some *Lambertia* species, while there was no significant effect of phosphite, trends were apparent: there was less colonisation of *P. cinnamomi* with increasing phosphite dose.
4. Phosphite was effective in the rare species
5. As stem inoculation bypasses the infection process it may be too severe a test and misses low levels of phosphite effectiveness that may be effective in the plant community environment.
6. Further testing of phosphite effectiveness is required using soil inoculation, which includes the normal infection process.
7. Need to understand the mechanisms of action of phosphite in order to understand phosphite effectiveness in different plant species.
8. The genus *Lambertia* is an ideal model to examine the mechanisms of action of phosphite.

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