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Influence of site and rate of low-volume aerial phosphite spray on lesion development of *Phytophthora cinnamomi* and phosphite persistence in *Lambertia inermis* var. *inermis* and *Banksia grandis*

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Abstract. The influence of site on effectiveness of low-volume spray of phosphite for the control of *Phytophthora cinnamomi* was compared between sites differing in soil nutrient status. The sites ranged from infertile deep grey sands to a red loam in the Esperance Plains bioregion, and infertile gravelly sand of the Havel site-vegetation type P to a red loam of Havel site-vegetation type Q in the northern Jarrah Forest bioregion. Following low-volume spray of 0, 24 or 48 kg phosphite/ha, phosphite effectiveness was determined from assessment of rate of colonisation and inhibition of stem colonisation by *P. cinnamomi*, and stem phosphite concentration in challenge inoculation stems of *Lambertia inermis* var. *inermis* in Esperance Plains bioregion sites and *Banksia grandis* in Jarrah Forest bioregion sites. Phosphite effectiveness was mainly influenced by plant species rather than site. Phosphite spray significantly controlled *P. cinnamomi* colonisation in *B. grandis*, but not in *L. inermis* var. *inermis*. Site had no consistent influence on the effect of phosphite on *P. cinnamomi* despite large differences in soil nutrient status between sites. Differences between sites and site ranking changed with inoculation time and rate of phosphite spray, and duplicate sites differed significantly from each other. Site, rate of phosphite spray, time after spray and plant species significantly affected stem phosphite concentrations. In both Esperance Plains bioregion and Jarrah Forest bioregion sites, stem phosphite concentrations were greatest in fertile loam sites and least in infertile sand or gravelly sand sites. Stem phosphite concentrations increased with rate of phosphite spray with concentrations in stems receiving 48 kg phosphite/ha being 1.2 to 3-times-greater than that in stem sprayed with 24 kg phosphite/ha. Six months after low-volume spray, phosphite concentrations in *L. inermis* var. *inermis* stems had declined for all sites and spray rates to only 4–17% of original concentrations. Decline of phosphite concentrations in *B. grandis* stems was slower than that for *L. inermis* var. *inermis* stems. The results indicate that effectiveness of phosphite against *P. cinnamomi* infection in different communities will depend more on plant species composition than soil nutrient status. Differences in phosphite effectiveness between plant species may be related to differences in phosphite concentration thresholds yet to be quantified, above which tissue concentrations must be achieved before inhibition of *P. cinnamomi* colonisation occurs. In rapid colonisation/phosphite low-responsive species such as *L. inermis* var. *inermis*, the threshold may be greater than that required for a response in a rapid colonisation/phosphite high-response species such as *B. grandis*. These results demonstrate the need to determine thresholds for individual plant species and in different environments.

Additional keywords: drought stress, integrated control strategies, phosphate status, phosphonate.

Introduction

Invasion of the introduced soil-borne multihost *Phytophthora cinnamomi* is a major threat to flora conservation in the South-West Botanical Province of Western Australia (Shearer *et al*. 2007*a*), an internationally recognised Biodiversity Hotspot (Myers *et al*. 2000). Current strategies applied within the province aim to conserve flora threatened by *P. cinnamomi* by integrating delaying tactics such as hygiene (Shearer and Tippett 1989), long-term *ex-situ* seed conservation (Cochrane *et al*. 2007), translocations (Monks and Coates 2002) and application of the systemic fungicide potassium phosphite that reduces the rate of disease development (Shearer and Tippett 1989; Barrett 2003; Shearer *et al*. 2004*b*; Shearer and Fairman 2007*a*, *b*).

Although rare and threatened flora growing on a variety of soil types are currently being sprayed with low-volume spray of phosphite (Barrett 2003), there has been no systematic determination of the influence of site on phosphite effectiveness for the control of *P. cinnamomi*. The efficacy of trunk injection (Shearer *et al*. 2004*b*, 2006; Shearer and Fairman 2007*a*), high-volume cover spray (Tynan *et al*. 2001; Shearer *et al*. 2004*b*; Shearer and Fairman 2007*b*) and low-volume spray (Komorek *et al*. 1997; Barrett 2001, 2003) has been investigated in sites ranging from deep sand to gravelly loam. Other than the

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observation of Barrett (2001) that variation in phosphite concentrations in *Taxandria spathulata* may be related to soil type, there has been little attempt to determine the influence of site or soil type on plant responses to phosphite spray. Such information is fundamental to the use of phosphite in integrated control strategies.

Ideally, effective control of *P. cinnamomi* by low-volume phosphite spray will depend on efficient uptake, long lasting action and a correlation between effective dose and inhibition of the pathogen. Phosphite uptake increased with increasing phosphite dosage applied, but varied greatly between plant species and environments (Komorek *et al*. 1997; Pilbeam *et al*. 2000; Barrett 2001; Tynan *et al*. 2001; Wilkinson *et al*. 2001). Phosphite concentrations recorded 15 days after high-volume cover spray of Jarrah Forest bioregion species with 5 g phosphite/L sprayed in spring varied between 25 to 200 μg/g for *Leucopogon verticillatus* and *Daviesia physodes* respectively (Tynan *et al*. 2001). Phosphite concentrations in stems of *Banksia grandis* grown in a glasshouse environment were 380 to 750 times greater than that in plants growing in the natural forest environment (Wilkinson *et al*. 2001). Phosphite concentrations declined rapidly in native species (Komorek *et al*. 1997; Pilbeam *et al*. 2000; Barrett 2001; Tynan *et al*. 2001), but control of *P. cinnamomi* has been observed in *B. grandis* even though no phosphite could be detected in the tissue (Tynan *et al*. 2001). Wilkinson *et al*. (2001) found a significant negative linear relationship between phosphite concentration and lesion development for *B. hookeriana* in a glasshouse. However, other than the general observation that an increase in spray rate decreased development of *P. cinnamomi* (Pilbeam *et al*. 2000; Barrett 2001; Tynan *et al*. 2001; Shearer *et al*. 2004*b*, 2006; Shearer and Fairman 2007*a*, *b*), no significant relationships have been reported between phosphite concentration and inhibition of lesion development of *P. cinnamomi* in plant species in native communities.

Phosphate is a competitive inhibitor of phosphite uptake *in vitro* (Barchietto *et al*. 1988) and *in planta* (Smillie *et al*. 1989; Carswell *et al*. 1996). Thus soil phosphorus status may affect phosphite effectiveness in different communities but this has yet to be determined. Soil properties and the phosphate status of the soil environment have rarely been considered in published studies testing the efficacy of phosphite against *Phytophthora* species in plants (Shearer and Fairman 2007*a*).

This study sought to compare the influence of site on phosphite effectiveness. Site influences on effectiveness of low-volume spray of phosphite for the control of *Phytophthora cinnamomi* were compared between sites of differing soil nutrient status in the Esperance Plains and Jarrah Forest bioregions of the South-West Botanical Province of Western Australia.

Methods

Sites

As there is no system of site-vegetation typing in the Esperance Plains bioregion of South-West Botanical Province of Western Australia, sites were chosen by the major soil groups (Schoknecht 2002). The sites chosen in the bioregion occurred on a fertility gradient from relatively infertile grey deep sands duplex (DS) to grey shallow sands duplex (SS), brown loam (BL) and relatively fertile red loam (RL). There were two replicate sites having DS soils (DS1 and DS2). All sites were healthy but directly threatened by *P. cinnamomi* infestation. They had dense stands of *Lambertia inermis* var. *inermis*, which is susceptible to *P. cinnamomi* and was used as a test species. Details on site location and characteristics are given in Table 1. Sites in the Esperance Plains bioregion occurred in the region between the Stirling Range National Park and the Fitzgerald River National Park. Sites were flat or convex with northerly or southerly aspects (Table 1).

The five sites chosen in northern Jarrah Forest bioregion occurred on a fertility gradient from the relatively infertile P Havel site-vegetation type (Havel 1975) to the S, T of intermediate fertility and relatively fertile Q type. There were two replicate S type sites (S1 and S2). All sites were healthy but directly threatened by *P. cinnamomi* infestation. They had dense stands of *B. grandis*, which is susceptible to *P. cinnamomi* and was used as a test species. Details on site location and characteristics are given in Table 1. Jarrah Forest bioregion sites occurred along the scarp on the western edge of the northern forest. All of the sites were convex in mid-slope positions (Table 1). Four of the sites had a southerly aspect with site S1 having an easterly aspect.

Table 1. Location and characteristics of Esperance Plains and Jarrah Forest bioregion sites of the South-West Botanical Province of Western Australia where plants were sprayed with phosphite to determine the effect of site differences on phosphite effectiveness against *Phytophthora cinnamomi*

Bioregion	Site[A]	Characteristic				
		Community	Longitude	Latitude	Local topography	Aspect
Esperance	DS1	*Lambertia* shrubland	34.1756°S	118.7186°E	Flat	North-east
Plains	DS2	*Lambertia* shrubland	34.5775°S	118.2106°E	Flat	South-east
	SS	*Lambertia* shrubland	34.5514°S	118.6228°E	Convex	South
	BL	*Lambertia* shrubland	34.3847°S	119.0972°E	Gentle slope	North-east
	RL	*Lambertia* shrubland	34.6144°S	118.6603°E	Convex mid-slope	South
Jarrah	P	Havel P	32.4250°S	116.0250°E	Convex mid-slope	South
Forest	S1	Havel S	32.5492°S	116.0183°E	Convex mid-slope	South-east
	S2	Havel S	32.7217°S	116.0269°E	Convex mid-slope	East
	T	Havel T	32.5492°S	116.0183°E	Convex mid-slope	South-east
	Q	Havel Q	32.7656°S	115.9831°E	Convex mid-slope	South

[A]Esperance Plains sites are by major soil group: DS = Grey Deep Sand Duplex; SS = Grey Shallow Sand Duplex; BL = Brown Loam; RL = Red Loam and in order of least (DS1) to most (RL) fertile. Jarrah forest sites are by Havel site-vegetation type P, S, T and Q and in order of least (P) to most (Q) fertile.

Experimental design

The independent variables were sites of differing soil nutrient status (5 sites in the Esperance Plains bioregion and 5 sites in the northern Jarrah Forest bioregion), inoculation time (3 for Esperance Plains bioregion sites and 4 for Jarrah Forest bioregion sites) and rate of low-volume spray of phosphite (0, 24 and 48 kg/ha). Soil properties, *P. cinnamomi* lesion development in inoculated stems and stem phosphite concentration were the dependent variables.

Phosphite treatments were replicated in a randomised block design. Treatments were replicated 15 times in DS, SS and RL sites and 10 times in the BL site in the Esperance Plains bioregion. Treatments were replicated 10 times in Jarrah Forest bioregion sites. For Esperance Plains bioregion sites, phosphite treatments were applied to 11 to 18-year-old small bushes of *Lambertia inermis* var. *inermis*, 1.2–4.4 m in height (mean ± s.e of 2.2 ± 0.03 m) on the 2–4 April 2001. One different branch of each bush was inoculated at one time. In Jarrah Forest bioregion sites, phosphite treatments were applied to small 7 to 11-year-old single-stemmed trees of *Banksia grandis*, 0.9–3.4 m in height (mean ± s.e of 1.7 ± 0.02 m) on the 17–18 April 2001. Sufficient *B. grandis* were sprayed in each block for a new tree to be inoculated each time. For Esperance Plains bioregion sites, Inoculation 1 was inoculated on 1 May 2001 and harvested on 12 June 2001; Inoculation 2 inoculated on 24 October 2001 and harvested on 12 November 2001 and Inoculation 3 inoculated on 9 April 2002 and harvested on 15 May 2002. For Jarrah Forest bioregion sites, Inoculation 1 was inoculated on 7 May 2001 and harvested on 27 June 2001; Inoculation 2 inoculated on 6 November 2001 and harvested on 27 November 2001; Inoculation 3 inoculated on 8 February 2002 and harvested on 26 February 2002 and Inoculation 4 inoculated on 7 May 2002 and harvested on 5 June 2002.

Phosphite spray

Phosphite solution was sprayed onto the foliage using a 'Microfit Herbi' (Micron Sprayers Ltd, Bromyard Herefordshire, UK) low-volume hand-held sprayer. The sprayer delivered a controlled droplet size of 250 µm at 1.78 mL/s 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 s per plant under still air conditions to give rates of spray 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 (Barrett 2003). Plants receiving no phosphite were sprayed with water and 0.2% surfactant. Plants were sprayed with phosphite on the 2–4 April 2001 for Esperance Plains bioregion sites and on the 17–18 April 2001 for Jarrah Forest bioregion sites.

Challenge inoculation

Agreement between results from stem inoculation and natural infection suggest that stem inoculation is a suitable bioassay for phosphite effectiveness against *P. cinnamomi* (Shearer and Fairman 2007*a*). One local isolate was selected from each of the bioregions for inoculation in the bioregion of origin. Isolate DP55 (WAC12892) was chosen for challenge inoculations in the Esperance Plains bioregion sites as it was isolated from an aggressive infestation in the Fitzgerald River National Park, it exhibited high sporulating ability and pathogenicity in previous studies (Shearer and Crane 2003), is used for the testing of the susceptibility of threatened flora to *P. cinnamomi* (Shearer *et al*. 2004*a*) and its pathogenicity was not statistically different from other *P. cinnamomi* isolates (Shearer *et al*. 2007*b*). Isolate SC72 (IMI264384) was chosen for challenge inoculations in the Jarrah Forest bioregion sites because it has been used in several previous studies in the Jarrah Forest bioregion (Shea *et al*. 1980; Tippett *et al*. 1985, 1987; Shearer *et al*. 1987*a*, *b*, 1988) and its pathogenicity was not statistically different from other *P. cinnamomi* isolates (Shearer *et al*. 1988). DP55 was isolated in 1996 from *B. baxteri* in the Bell Track *P. cinnamomi* infestation in the Fitzgerald River National Park, is A1 mating type and has an *in vitro* EC_{50} (50% inhibition of mycelial growth) to phosphite of 145 ± 24 µg phosphite/mL. SC72 was isolated from *Hibbertia subvaginata* at Ravenswood, Mandurah in 1965, is A2 mating type and has an *in vitro* EC_{50} to phosphite of 19 ± 2 µg phosphite/mL. Cultures were stored in water (Boesewinkel 1976) and grown on BBL Cornmeal medium (17 g/L distilled water; Becton, Dickson and Co.).

For challenge inoculation, branches of *L. inermis* var. *inermis*, 3–18 mm in diameter (mean ± s.e of 6.9 ± 0.1 mm) and stems of *B. grandis*, 8–32 mm in diameter (mean ± s.e of 15.5 ± 0.2 mm) were wound inoculated with *P. cinnamomi*. A cornmeal agar disk containing mycelium was bound to a fresh cut in the phloem as described in Shearer *et al*. (1988). Branches of *L. inermis* var. *inermis* were inoculated 0.5 m from the main stem, and stems of *B. grandis* were inoculated 0.5 m above soil level. Blank inoculations were made in a similar manner using sterile agar disks.

Assessment

Phytotoxicity

Phytotoxicity was assessed 4 weeks after phosphite spray using the phytotoxicity rating system of Barrett *et al*. (2002): 0 = no phytotoxicity, 1 = 1–20% foliage affected, 2 = 21–40% foliage affected, 3 = 41–60% foliage affected, 4 = 61–80% foliage affected and 5 = 81–100% foliage affected. Ratings were then divided into classes: 0, no symptoms; $0 < \times < 1$, mild phytotoxicity; $1 < \times < 2$ moderate phytotoxicity; $2 < \times < 3$, moderately severe phytotoxicity; $3 < \times < 4$ severe phytotoxicity and $4 < \times < 5$ extreme phytotoxicity.

Lesion

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.

Colonisation was determined by cutting stems above and below the inoculation point into 0.5-cm-long sections along the lesion and extending 10–15 cm into apparently healthy tissue. Healthy tissue was cut and plated first, with utensils sterilised between cuts and plating. Sections were plated sequentially 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 at least 2 days and the number infected with *P. cinnamomi* determined. Colonisation was defined as the length of section infected with *P. cinnamomi.*

Soil and tissue analysis

Soil properties

In April 2001, 2 samples were taken from the top 3 cm of soil at each end and in the middle of each site, and the soil sieved through a 2 mm sieve, dried at 40°C for 48 h and analysed using the following methods. Phosphorus content was determined colourimetrically using the method of Murphy and Riley (1962). Using the same solution, potassium content was determined with a flame photometer. The organic carbon content was determined using the Walkley-Black method (Piper 1942). Soil nitrogen was determined by extracting the soil with 1M potassium chloride solution and using the Kjeldahl method of McKenzie and Wallace (1954). The pH was determined from a 1 : 5 solution of soil and water. Percentage by weight of coarse (200–2000 µm) sand, fine (20–200 µm) sand, silt (2–20 µm) and clay (<2 µm) of the fine fraction were determined by the pipette method (Day 1965).

Soil moisture

At inoculation and harvest a sample was taken from the top 3 cm of soil at each end and in the middle of each site. The soil was sieved through a 2 mm sieve and a sample of moist and dried soil weighed before and after drying at 105°C for 24 h. Moisture was calculated as percent by weight of dried soil.

Tissue phosphite and phosphorus

Stem samples were washed in a 1% solution of Deconex 15-E® phosphate-free detergent (Borer Chemical Ltd Switzerland) and dried at 40°C for several days. An electric grinder with a 1-mm sieve was used to grind dried samples, the grinder being cleaned with compressed air and a fine brush between samples. One 2 g sample of ground material was placed in a screw-cap container 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).

Newly produced leaves from each replicate not sprayed with phosphite were washed, dried and ground as for phosphite analysis. Phosphorus content was determined colourimetrically using the method of Murphy and Riley (1962).

Meteorological data

For the Esperance Plains bioregion sites, air temperature and rainfall data were obtained from automated stations of the Western Australia Department of Agriculture at Many Peaks (29 km south of DS2), Wellstead (7 and 15 km south-east of SS and RL, respectively) and Jerramungup (49 km east of DS1 and 71 km north of BL). Data for Jarrah Forest bioregion sites was obtained from stations of the Western Australia Meteorological Bureau at Karnet (5 km north of P and 13 km north of S1 and T), South Dandalup (11 km north of S1 and T) and Dwellingup (3 km east of S2 and 9 km north-east of Q).

Analysis

Rate of colonisation at time 't' was calculated as:

$$\text{Rate of colonisation}_t = (\text{Total colonisation above and below inoculation point}/2)_t/(\text{Days from inoculation}_t \text{ to harvest}_t).$$

In order to correct for different environmental conditions at each time of challenge inoculation, inhibition at time 't' was calculated for colonisation in phosphite sprayed stems in relation to colonisation in stems not sprayed with phosphite for each replicate:

$$\%\ \text{Colonisation inhibition}_t = ((\text{Not sprayed colonisation}_t - \text{Sprayed colonisation}_t)/\text{Not sprayed colonisation}_t) \times 100$$

Assumptions of normality were checked by plotting residuals (Kirby 1993). Data were transformed to logarithms, and percentage data transformed to arcsin square root values to homogenise the variance. For percentage inhibition, the data for the no-phosphite treatment were removed before Analysis of Variance (ANOVA) because of the large number of zero values affecting assumptions of normality. Although repeated-measurements were made on the same *L. inermis* var. *inermis* plants, different branches were sampled on each occasion. For *B. grandis*, repeated-measurements were made on the same plots, but different plants harvested. The repeated-measures design was analysed using the split-unit model (Mead 1988). Site, inoculation time and rate of phosphite were fixed factors, and replicates nested within sites were considered a random effect in the ANOVA. This approach reduces random variation by sampling the same plants or plants from the same plots. Correlation between repeated-measures was incorporated in the analysis by using the split-unit approach and including the replicate-nested-within-site term. More than one measurement from the same plant or plot was corrected for testing multiple hypotheses by applying the strict Bonferroni correction, using $\alpha = 0.025$. The Pearson correlation coefficient was calculated for a measure of the linear association between variables. The Bonferroni correction was not applied to correlation coefficients as recommended by Moran (2003). Significance was determined at $P \leq 0.05$.

Results

Sites

The test species in the Esperance Plains bioregion was identified as *L. inermis* var. *inermis* and voucher specimens have been deposited at the Western Australia Herbarium (PERTH 06170102, 06170110, 06170129, 06170137 and 06170145). *Lambertia inermis* var. *inermis* was a dominant component in each site. *Adenanthos cuneatus* and *Banksia* species mainly occurred on the sandy sites and two *Beaufortia* species were only on the loams.

The test species *B. grandis* was a dominant component in each of the Jarrah Forest bioregion sites. The infertile P type occurred on gravelly sand in mid-slope positions. It was characterised by the relative absence of *Macrozamia riedlei*, *Leucopogon capitellatus* and *Phyllanthus calycinus* in the understorey and frequent occurrence of *Allocasuarina fraseriana* in the overstorey. Type S is the broadest and most common type on laterite-mantled Jarrah Forest bioregion uplands (Havel 1975). The chief indicators were *B. grandis* and *Persoonia longifolia* in the second storey with *Adenanthos barbiger*, *Hovea chorizemifolia* and *L. capitellatus*, *P. calycinus* in the shrub stratum. The T type shared with type S *L. capitellatus* and *P. calycinus*, with the addition of *Acacia urophylla*, *Clematis pubescens* and *Pteridium esculentum*. Type Q is associated with fertile red loams on slopes of major river valleys in the western high rainfall zone of the Jarrah Forest bioregion (Havel 1975). Chief indicators were *A. extensa*, *L. capitellatus*, *P. calycinus* with *Lasiopetalum floribundum* and *P. esculentum.* In previous research, *Isopogon sphaerocephalus* has been an indicator of high impact of *P. cinnamomi* in sites with an impervious sub-surface layer (Shearer unpublished data). *Isopogon sphaerocephalus* occurred in the P and Q sites, indicating an impervious sub-surface layer in both of these sites.

Soil characteristics

Coarse sand was most significant in the deep sand at site DS1, and least significant in the red loam (RL) and the shallow sand at SS (Table 2). Coarse sand in the BL site was intermediate between RL and DS1. Fine sand was the converse of coarse sand (Table 2) and silt did not differ significantly between sites. There was no significant difference in clay content between sites. Soil pH was mainly in the range 5.1–5.5 (Table 2). Bare ground was significantly less in the relatively fertile loam sites and SS, greatest in DS1, and intermediate for DS2 (Table 2).

Soil of Havel site-vegetation type Q had the lowest proportion of coarse sand, significantly lower than all other sites (Table 2). There was no significant difference for fine sand, silt and clay between sites, but soil at the Q site had greater silt and clay than the other sites (Table 2). Soil pH was similar for all Jarrah Forest bioregion sites being in the range 5.3–5.4. Bare ground was significantly less in the relative fertile Q than the other sites (Table 2).

For Esperance Plains bioregion sites, the phosphorus in the loams was significantly greater than in the sands (Fig. 1). Extractable phosphorus was <0.01 μg/g in all sites. There was significantly more potassium in the loams and shallow sands than in the deep sands. Nitrogen and organic carbon was significantly greater in the loams than in the deep sands. Leaf phosphorus was significantly greater in new leaves of *L. inermis* var. *inermis* in the Esperance Plains bioregion sites than in new leaves of *B. grandis* in Jarrah Forest bioregion sites (Fig. 1). Leaf phosphorus was least for DS1 and BL, while DS2 and RL sites had the greatest leaf phosphorus. There was no significant linear relationship between soil and leaf phosphorus for Esperance Plains bioregion sites.

For Jarrah Forest bioregion sites, totals for phosphorus, potassium, nitrogen and organic carbon values were significantly greater in the Q than the other sites (Fig. 1). No significant differences for these nutrients occurred between sites other than at Q, except for phosphorus which was least in the P site (Fig. 1). Extractable phosphorus was <0.01 μg/g in all sites. Within the Jarrah Forest bioregion there was a positive gradient in *B. grandis* leaf phosphorus values - from the least in site type P to the greatest in the Q site (Fig. 1). There was a significant linear relationship between soil and leaf phosphorus:

$$B.\ grandis\ \text{Leaf Phosphorus} = 5.056 + 0.178\ \text{Soil Phosphorus}\ (R^2 = 0.84, P = 0.03).$$

Soil moisture, rainfall and air temperature

For the Esperance Plains bioregion, percentage soil moisture tended to be greatest in loamy soil sites BL and RL, and least in deep sand sites (DS1 & DS2) (Fig. 2*a*). Soil moisture increased rapidly during the first inoculation period and decreased rapidly in the second inoculation period. Drought deaths were observed in site DS1 at the time of the second inoculation.

Table 2. Soil properties (mean ± s.e.) of Esperance Plains and Jarrah Forest bioregion sites of the South-West Botanical Province of Western Australia where plants were sprayed with phosphite to determine the effect of site differences on phosphite effectiveness against *Phytophthora cinnamomi*

Bioregion	Site[A]	Property							
		Soil type	Colour	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	pH	Bare ground (%)
Esperance Plains	DS1	Sand	Grey	76.0 ± 2.0	23 ± 2	0.4 ± 0.3	1.0 ± 0.3	5.20 ± 0.05	58 ± 4
	DS2	Sand	Brown	31.0 ± 2.0	66 ± 1	1.0 ± 0.5	1.0 ± 0.5	5.50 ± 0.10	31 ± 4
	SS	Sand	Brown	11.0 ± 0.5	85 ± 2	2.0 ± 1.0	2.0 ± 1.0	5.30 ± 0.05	12 ± 2
	BL	Loam	Brown	31.0 ± 3.0	63 ± 3	2.0 ± 1.0	3.0 ± 1.0	5.10 ± 0.10	5 ± 1
	RL	Loam	Red	9.0 ± 1.0	90 ± 1	0.4 ± 0.1	1.0 ± 0.2	5.10 ± 0.20	14 ± 3
Jarrah Forest	P	Laterite	Brown	53.0 ± 2.0	39 ± 3	4.0 ± 2.0	4.0 ± 2.0	5.30 ± 0.10	42 ± 3
	S1	Laterite	Brown	56.0 ± 1.0	39 ± 2	2.0 ± 1.0	3.0 ± 1.0	5.30 ± 0.06	50 ± 3
	S2	Laterite	Brown	50.0 ± 2.0	45 ± 4	3.0 ± 1.0	3.0 ± 1.0	5.30 ± 0.02	53 ± 3
	T	Laterite	Brown	57.0 ± 3.0	38 ± 4	3.0 ± 1.0	2.0 ± 1.0	5.40 ± 0.10	49 ± 7
	Q	Gravel-loam	Brown	44.0 ± 1.0	43 ± 4	7.0 ± 2.0	7.0 ± 3.0	5.30 ± 0.04	18 ± 3

[A]Esperance Plains sites are by major soil group: DS = Grey Deep Sand Duplex; SS = Grey Shallow Sand Duplex; BL = Brown Loam; RL = Red Loam and in order of least (DS1) to most (RL) fertile. Jarrah Forest sites are by Havel site-Vegetation type P, S, T and Q and in order of least (P) to most (Q) fertile. Soil taken from the top 3 cm.

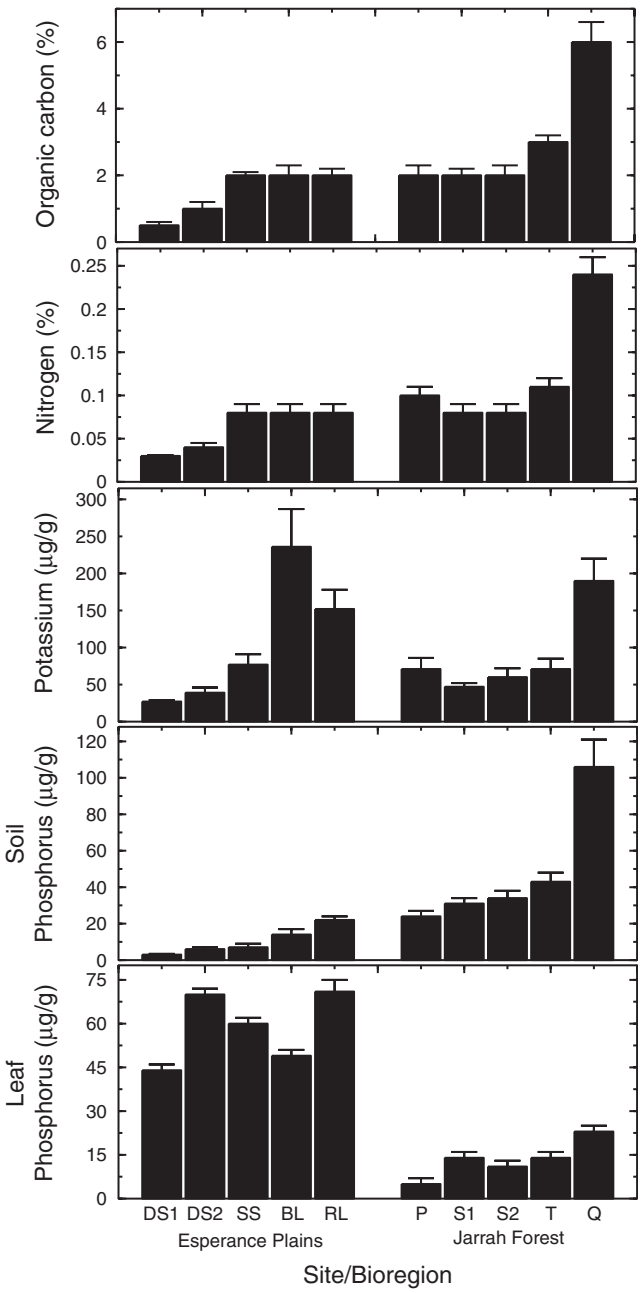

Fig. 1. Changes in mean (±s.e.) leaf phosphorus and soil phosphorus, potassium, nitrogen and organic carbon in the top 3 cm of soil from five sites in each of the Esperance Plains and Jarrah Forest bioregions of the South-West Botanical Province of Western Australia. Esperance Plains bioregion sites are categorised by major soil group: DS1 and 2 = Grey Deep Sand Duplex; SS = Grey Shallow Sand Duplex; BL = Brown Loam; RL = Red Loam and in order of least (DS1) to most (RL) fertile. Jarrah Forest bioregion sites are by Havel site-vegetation types P, S, T and Q and in order of least (P) to most (Q) fertile.

Mean minimum and maximum temperature during the three inoculation periods for the Esperance Plains bioregion sites did not differ greatly between meteorological stations (Table 3). Lowest minimum temperatures occurred during the first and second inoculations with greatest minimum temperatures occurring at the time of the third inoculation. The maximum temperature was lowest during the first inoculation and highest at the time of the third inoculation. Greater variability in rainfall between stations was more significant than temperature differences (Table 3). The least duration and amount of rainfall occurred at the time of the second inoculation period.

For Jarrah Forest bioregion sites, soil moisture was greatest for the Q type during the first inoculation period (Fig. 2*b*). In other inoculation periods there were no consistent differences between sites. Soil moisture was lowest at the time of the third inoculation period and drought deaths were observed in P and Q type sites. No *P. cinnamomi* was found in the roots of sampled dead plants.

As was the case for the Esperance Plains bioregion, mean minimum and maximum temperatures did not differ greatly between Jarrah Forest bioregion meteorological stations during the inoculation periods (Table 3). Minimum and maximum temperatures were least in the first inoculation and greatest in the second and third inoculations. Rainfall duration and amount was more varied between stations than temperature (Table 3). The greatest duration and amount of rainfall occurred during the first and fourth inoculations.

Phytotoxicity

Phytotoxicity was evident in *L. inermis* var. *inermis* as bleaching of individual leaflets which were shed 1–4 months after spraying. Phytotoxicity was mild to moderate with ratings 2–3 times greater for plants sprayed with 48 kg phosphite/ha than those sprayed with 24 kg phosphite/ha (Table 4). There was no strong association between rating and site type. The two grey deep sand sites showed divergent ratings with DS1 having lowest ratings and DS2 the highest ratings.

Phytotoxicity was evident in *B. grandis* as an orange-brown blotching on part of the leaf. Only a few leaves per plant showed the blotching and affected leaves were retained on the plant. Phytotoxicity was mild to moderate with no significant differences between sites and phosphite rate (Table 4).

Lesion development

Phytophthora cinnamomi development in inoculated stems was assessed from visible lesion development, tangential spread, linear spread above and below the inoculation point and total lesion length and colonisation above and below the inoculation point determined from plating tissue. The various assessments of pathogen development were highly correlated with each other with r = 0.83–0.99 ($P < 0.01$, n = 45) for Esperance Plains bioregion sites and r = 0.88–0.99 ($P < 0.01$, n = 60) for Jarrah Forest bioregion sites.

Rate of colonisation and percentage inhibition were chosen for assessment of pathogen development. The rate of colonisation compensates for differences in the period from inoculation to harvest. Percentage inhibition (i.e. colonisation in either phosphite treatment expressed as a percentage of the not sprayed replicate) compensates for different environmental conditions between inoculation times.

Rate of colonisation

Inoculation time was the major factor affecting rate of *P. cinnamomi* colonisation in inoculated stems of *L. inermis*

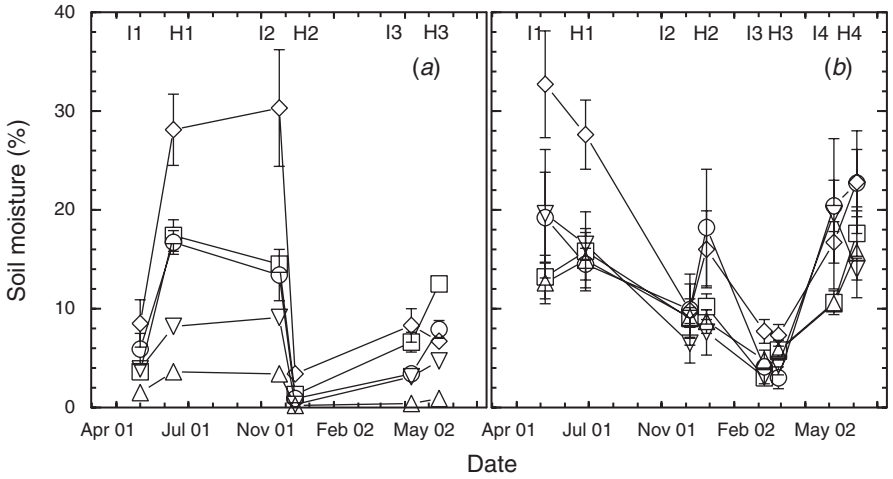

Fig. 2. Mean percentage soil moisture (±s.e.) at: (*a*) inoculation (I1–3) and harvest (H1–3) in 5 sites in the Esperance Plains bioregion in which *Lambertia inermis* var. *inermis* was not sprayed or sprayed with phosphite and inoculated with *Phytophthora cinnamomi*. Esperance Plains bioregion sites are categorised by major soil group: Grey Deep Sand Duplex – DS1 (△) and DS2 (▽); Grey Shallow Sand Duplex – SS (○); Brown Loam – BL (□); Red Loam – RL (◇), and (*b*) at inoculation (I1–4) and harvest (H1–4) in 5 sites in Jarrah Forest bioregion of the South-West Botanical Province of Western Australia in which *Banksia grandis* was not sprayed, or sprayed with phosphite and inoculated with *P. cinnamomi*. Jarrah Forest bioregion sites by major Havel vegetation type: P (○); S1(△) and S2 (▽); T (□) and Q (◇). Inoculation/Harvest dates: (*a*) Inoculation 1 inoculated on 1 May 2001 and harvested on 12 June 2001; Inoculation 2 inoculated on 24 October 2001 and harvested on 12 November 2001; Inoculation 3 inoculated on 9 April 2002 and harvested on 15 May 2002. (*b*) Inoculation 1 inoculated on 7 May 2001 and harvested on 27 June 2001; Inoculation 2 inoculated on 6 November 2001 and harvested on 27 November 2001; Inoculation 3 inoculated on 8 February 2002 and harvested on 26 February 2002; Inoculation 4 inoculated on 7 May 2002 and harvested on 5 June 2002.

var. *inermis* in Esperance Plains bioregion sites (Table 5). Rate of colonisation did not differ significantly between sites (Table 5), and is presented averaged over sites in Fig. 3*a*. The greatest rate of colonisation occurred in the second autumn inoculation in April–May 2002, and the least in the first autumn inoculation of May 2001. Low-volume spray of phosphite had no significant or consistent effect on rate of colonisation in *L. inermis* var. *inermis* (Fig. 3*a*).

Inoculation time and rate of phosphite spray were the major factors affecting the rate of *P. cinnamomi* colonisation in inoculated stems of *B. grandis* in Jarrah Forest bioregion sites (Table 5). Colonisation did not differ significantly between sites (Table 5) and is presented averaged over sites in Fig. 3*b*. For all Jarrah Forest bioregion sites, the rate of colonisation of *P. cinnamomi* in *B. grandis* stems was greatest in the spring inoculation of November 2001 (second inoculation), and least at the first autumn inoculation of May 2001 for each site and rate of phosphite spray (Fig. 3*b*).

In contrast to *L. inermis* var. *inermis* in the Esperance Plains bioregion, colonisation of *B. grandis* by *P. cinnamomi* was significantly affected by low-volume spray of phosphite (Table 5, Fig. 3*b*). Colonisation of phosphite sprayed stems was significantly less than that of not sprayed stems at all inoculation times. In the second and third inoculations, less colonisation occurred in stems sprayed with 48 kg phosphite/ha than those sprayed with 24 kg phosphite/ha.

Inhibition of colonisation

Inhibition of *P. cinnamomi* colonisation of *L. inermis* var. *inermis* stems by phosphite differed significantly between sites (Table 5), with the BL site having the greatest or second greatest inhibition at both rates of phosphite spray whislt the RL site had the least inhibition (Fig. 4). There were no significant differences between the two DS sites. Except for the SS and BL sites, inhibition significantly (Table 5) decreased with time from 19–43%, 0.2 years after spraying to 5–30% inhibition 0.6–1.1 years after spraying (Fig. 4). Phosphite spray had no significant or consistent effect on inhibition of colonisation in *L. inermis* var. *inermis* stems (Fig. 4).

Although inhibition of *P. cinnamomi* colonisation of *B. grandis* stems by phosphite differed significantly between Jarrah Forest bioregion sites (Table 5), there was no consistent difference between sites (Fig. 5). Site ranking changed within inoculation time and phosphite rate. Both S sites differed significantly from each other for both phosphite treatments 0.2 years after spraying, and for the 24 kg phosphite/ha 0.8 years after spraying. For all, except the Q site, inhibition was greatest (43–88%) 0.2 years after spray, declining to 19–42% by 1.1 years after spraying (Fig. 5). Inhibition at 0.6–0.8 years after spraying was intermediate between values for 0.2 and 1.1 years after spraying. Overall, rate of phosphite spray significantly affected inhibition of *P. cinnamomi* colonisation (Table 5); spraying with 48 kg phosphite/ha causing greater

Table 3. Mean (±s.e.) minimum and maximum temperature (°C), number of rain days and total rainfall (mm) for the period from inoculation to harvest at either three inoculation times for meteorological stations near sites in the Esperance Plains bioregion, or four inoculation times for meteorological stations near sites in the Jarrah Forest bioregion where plants were sprayed with phosphite to determine the effect of site differences on phosphite effectiveness against *Phytophthora cinnamomi*

Bioregion	Environment variable	Meteorological station	Associated site[A]	Inoculation[B] 1	2	3	4
Esperance Plains	Minimum temperature (°C)	Many Peaks	DS2	8.1 ± 0.3	8.1 ± 0.5	11.4 ± 0.6	
		Wellstead	SS, RL	8.2 ± 0.4	8.6 ± 0.6	12.0 ± 0.6	
		Jerramungup	DS1, BL	7.7 ± 0.4	7.4 ± 0.8	11.4 ± 0.7	
	Maximum temperature (°C)	Many Peaks	DS2	17.3 ± 0.6	17.7 ± 0.7	20.4 ± 0.6	
		Wellstead	SS, RL	18.4 ± 0.6	19.3 ± 0.9	22.0 ± 0.7	
		Jerramungup	DS1, BL	17.4 ± 0.6	22.5 ± 1.0	22.2 ± 0.7	
	Number rain days	Many Peaks	DS2	29.0	7.0	15.0	
		Wellstead	SS, RL	13.0	3.0	10.0	
		Jerramungup	DS1, BL	24.0	5.0	13.0	
	Total rainfall (mm)	Many Peaks	DS2	145.8	6.4	90.4	
		Wellstead	SS, RL	38.6	7.2	60.8	
		Jerramungup	DS1, BL	44.8	1.6	23.2	
Jarrah Forest	Minimum temperature (°C)	Karnet	P, S1,T	7.4 ± 0.4	11.2 ± 0.6	12.8 ± 0.7	8.3 ± 0.4
		Dwellingup	S2,Q	6.4 ± 0.5	11.4 ± 0.5	12.6 ± 0.4	7.9 ± 0.7
	Maximum temperature (°C)	Karnet	P, S1,T	17.6 ± 0.4	25.4 ± 0.8	29.4 ± 1.0	19.3 ± 0.5
		Dwellingup	S2,Q	17.5 ± 0.4	25.4 ± 0.9	29.3 ± 1.1	19.1 ± 0.4
	Number rain days	Karnet	P	23.0	3.0	2.0	12.0
		S Dandalup Dam	S1,T	31.0	4.0	2.0	14.0
		Dwellingup	S2,Q	28.0	4.0	3.0	20.0
	Total rainfall (mm)	Karnet	P	138.2	25.6	1.4	176.7
		S Dandalup Dam	S1,T	164.5	38.7	2.0	204.4
		Dwellingup	S2,Q	147.3	48.4	2.2	214.0

[A]Esperance Plains bioregion sites are by major soil group: DS = Grey Deep Sand Duplex; SS = Grey Shallow Sand Duplex; BL = Brown Loam; RL = Red Loam. Jarrah Forest bioregion sites are by Havel site-vegetation type P, S, T and Q.

[B]Esperance Plains bioregion sites: Inoculation 1 inoculated on 1 May 2001 and harvested on 12 June 2001; Inoculation 2 inoculated on 24 October 2001 and harvested on 12 November 2001; Inoculation 3 inoculated on 9 April 2002 and harvested on 15 May 2002. Jarrah Forest bioregion sites: Inoculation 1 inoculated on 7 May 2001 and harvested on 27 June 2001; Inoculation 2 inoculated on 6 November 2001 and harvested on 27 November 2001; Inoculation 3 inoculated on 8 February 2002 and harvested on 26 February 2002; Inoculation 4 inoculated on 7 May 2002 and harvested on 5 June 2002.

Table 4. Mean phytotoxicity ratings (±s.e.) 4 weeks after low-volume spray of 24 and 48 kg/ha phosphite of either *Lambertia inermis* var. *inermis* or *Banksia grandis* in five sites in the Esperance Plains or Jarrah Forest bioregions of the South-West Botanical Province of Western Australia

Bioregion (host)	Site[A]	Low volume phosphite spray (kg/ha) 0	24	48
Esperance Plains	DS1	0	0.4 ± 0.1[B]	1.2 ± 0.2
(*L. inermis*	DS2	0	1.3 ± 0.2	2.1 ± 0.2
var. *inermis*)	SS	0	1.1 ± 0.1	1.8 ± 0.2
	BL	0	0.9 ± 0.1	1.5 ± 0.2
	RL	0	0.8 ± 0.1	1.4 ± 0.2
Jarrah Forest	P	0	1.3 ± 0.2	1.6 ± 0.2
(*B. grandis*)	S1	0	1.2 ± 0.1	1.6 ± 0.1
	S2	0	1.1 ± 0.1	1.2 ± 0.1
	T	0	1.1 ± 0.1	1.4 ± 0.1
	Q	0	1.2 ± 0.2	1.6 ± 0.2

[A]Sites are by major soil group: DS = Grey Deep Sand Duplex; SS = Grey Shallow Sand Duplex; BL = Brown Loam; RL = Red Loam for the Esperance Plains bioregion and by Havel site-vegetation type P, S, T and Q for the Jarrah Forest bioregion.

[B]Phytotoxicity ratings after Barrett *et al*. (2002): 0, no symptoms; $0 < \times < 1$, mild phytotoxicity; $1 < \times < 2$ moderate phytotoxicity; $2 < \times > 3$, moderately severe phytotoxicity.

inhibition of colonisation than 24 kg phosphite/ha for most sites (Fig. 5).

Phosphite concentrations

Site and time after spray and rate of phosphite spray significantly affected phosphite concentrations in *L. inermis* var. *inermis* in Esperance Plains bioregion sites (Table 5). Figure 6 plots time after spray of stem phosphite concentrations against site and rate of phosphite spray. For DS1 and BL, stems sprayed with 48 kg phosphite/ha were up to twice the concentrations in stems sprayed with 24 kg phosphite/ha (Fig. 6). For all sites except RL with 24 kg phosphite/ha, phosphite concentrations in *L. inermis* var. *inermis* stems were greatest 0.2 years after spraying and declined rapidly by 0.6–1.1 years after spray (Fig. 6). Phosphite concentrations were consistently greatest in stems of *L. inermis* var. *inermis* in the RL site (Fig. 6). With the exception of inoculation 1 for DS1, phosphite concentrations were least in the DS and SS sites. The concentrations for the BL site were intermediate between the RL and sand sites. The duplicate DS sites differed significantly from each other for both rates of phosphite spray and time after sprays except the 48 kg phosphite/ha at inoculation 2 (Fig. 6).

Table 5. Degrees of freedom (df) and Mean Square (MS) from analysis of variance of rate of colonisation and percentage inhibition of *Phytophthora cinnamomi* and phosphite concentration in stems of either *Lambertia inermis* var. *inermis* or *Banksia grandis* not sprayed or sprayed with 24 or 48 kg phosphite/ha in 5 sites in either the Esperance Plains bioregion and assessed at three times or the Jarrah Forest bioregion and assessed at 4 times, respectively

Bioregion (host)	Source of variation	Rate of colonisation		Percent inhibition		Phosphite concentration	
		df	MS	df	MS	df	MS
Esperance Plains	Site	4	0.58	4	0.76**	4	47.65**
(*L. inermis* var.	Inoculation time	2	193.62**[A]	2	0.87**	2	554.55**
inermis)	Rate phosphite	2	0.36	1	0.27	1	3.51
	Rate × Site	8	0.40	4	0.01	4	2.42
	Rate × Inoc.	4	0.35	2	0.18	2	7.09
	Inoc × Site	8	4.60**	8	0.11	8	29.46**
	Rate × Site × Inoc.	16	0.16	8	0.02	8	2.20
	Rep(Site)	66	0.53**	65	0.26**	66	6.05**
	Error	490	0.32	289	0.12	314	3.70
Jarrah Forest	Site	4	0.88	4	0.63**	4	25.43**
(*B. grandis*)	Inoculation time	3	143.38**	3	3.03**	3	48.48**
	Rate phosphite	2	45.49**	1	0.77*	1	32.42**
	Rate × Site	8	1.00*	4	0.09	4	0.79
	Rate × Inoc.	6	3.35**	3	0.05	3	1.27
	Inoc × Site	12	2.03**	12	0.31	12	1.25
	Rate × Site × Inoc.	24	0.55	12	0.08	12	0.90
	Rep(Site)	45	0.52	45	0.18	45	1.01
	Error	433	0.41	247	0.15	306	0.76

[A]Level of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$.

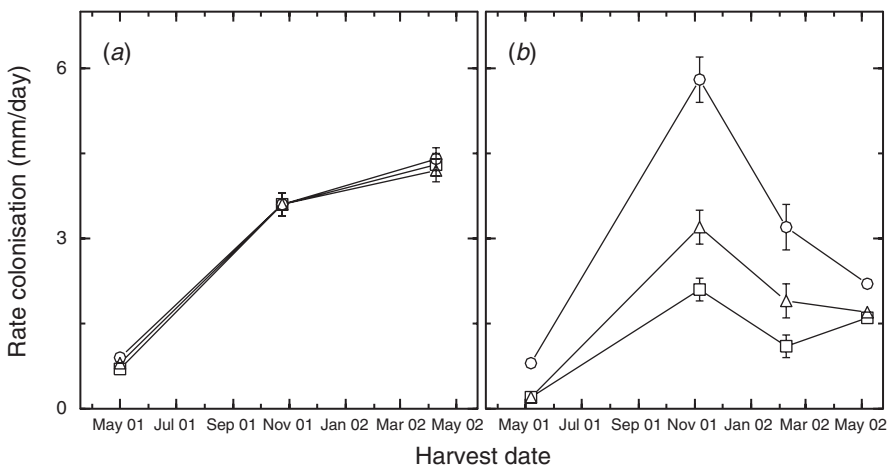

Fig. 3. Mean rate of *Phytophthora cinnamomi* colonisation (±s.e.) in (*a*) *Lambertia inermis* var. *inermis* stems inoculated at three times following either not sprayed (○) or low-volume spray of 24 (△) or 48 (□) kg phosphite/ha in 5 sites in the Esperance Plains bioregion and (*b*) *Banksia grandis* stems inoculated at four times following either not sprayed (○) or low-volume spray of 24 (△) or 48 (□) kg phosphite/ha in 5 sites in the Jarrah Forest bioregion of the South-West Botanical Province of Western Australia.

Sites, time after spray and rate of phosphite spray had a significant effect on phosphite concentrations in *B. grandis* in Jarrah Forest bioregion sites, similar to the case for *L. inermis* var. *inermis* in the Esperance Plains bioregion (Table 5). Figure 7 shows the change with time after spray of stem phosphite concentrations against site and rate of phosphite spray for this bioregion. Phosphite concentrations were 2–8 times greater in *B. grandis* stems than in *L. inermis* var. *inermis* stems (Figs 6 and 7). With the exception of the P type 1.1 years after spray, phosphite concentrations were 1.2–3 times greater for *B. grandis* stems sprayed with 48 kg phosphite/ha than those sprayed with 24 kg/phosphite (Fig. 7). For all sites, phosphite concentrations declined gradually with time after spraying (Fig. 7). Similar to the Esperance Plains bioregion, the greatest or second greatest phosphite concentrations occurred in *B. grandis* stems in the red loam Havel vegetation type Q

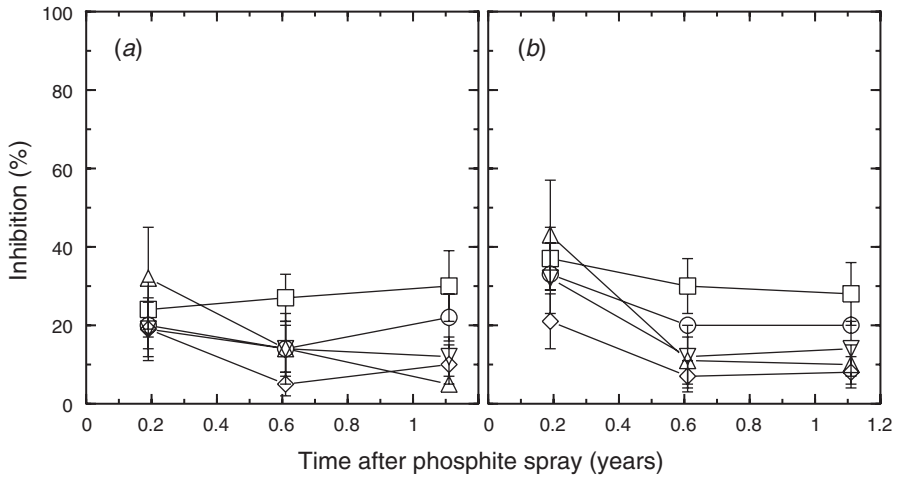

Fig. 4. Effect of years after low-volume phosphite spray and rate of (*a*) 24 and (*b*) 48 kg phosphite/ha on mean percent inhibition (±s.e.) of *Phytophthora cinnamomi* in stems of *Lambertia inermis* var. *inermis* in five sites in Esperance Plains bioregion of the South-West Botanical Province of Western Australia. Esperance Plains bioregion sites are by major soil group: Grey Deep Sand Duplex – DS1 (△) and DS2 (▽); Grey Shallow Sand Duplex – SS (○); Brown Loam – BL (□); Red Loam – RL (◇). Inoculation/Harvest dates for Esperance Plains bioregion sites: Inoculation 1 inoculated on 1 May 2001 and harvested on 12 June 2001; Inoculation 2 inoculated on 24 October 2001 and harvested on 12 November 2001; Inoculation 3 inoculated on 9 April 2002 and harvested on 15 May 2002.

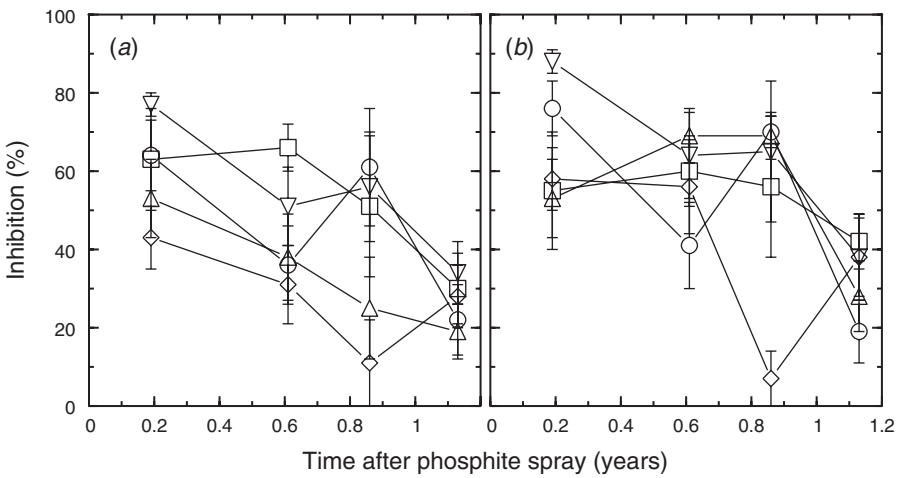

Fig. 5. Effect of years after low-volume phosphite spray and rate of (*a*) 24 and (*b*) 48 kg phosphite/ha on mean percent inhibition (±s.e.) of *Phytophthora cinnamomi* in stems of *Banksia grandis* in five sites in the Jarrah Forest bioregion of the South-West Botanical Province of Western Australia. Jarrah Forest bioregion sites by major Havel vegetation type: P (○); S1(△) and S2 (▽); T (□) and Q (◇). Inoculation/Harvest dates Jarrah Forest bioregion sites: Inoculation 1 inoculated on 7 May 2001 and harvested on 27 June 2001; Inoculation 2 inoculated on 6 November 2001 and harvested on 27 November 2001; Inoculation 3 inoculated on 8 February 2002 and harvested on 26 February 2002; Inoculation 4 inoculated on 7 May 2002 and harvested on 5 June 2002.

(Fig. 7). Lowest phosphite concentrations occurred in *B. grandis* in the infertile P type. At 0.6–0.8 years after spraying, phosphite concentrations for the S and T type sites were intermediate between those for the Q and P. Concentrations for both S sites differed significantly from each other for 24 kg phosphite/ha 0.2 years after spraying and both phosphite rates 0.6–0.8 years after spraying (Fig. 7).

Phosphite persistence

By half a year after low-volume spray, phosphite concentrations in *L. inermis* var. *inermis* stems for all sites and spray rates had declined to only 4–17% of original concentrations (Fig. 6). By 1.1 years after spraying, phosphite concentrations had levelled off at very low concentrations, except the red loam sprayed with 24 kg of phosphite/ha (Fig. 6).

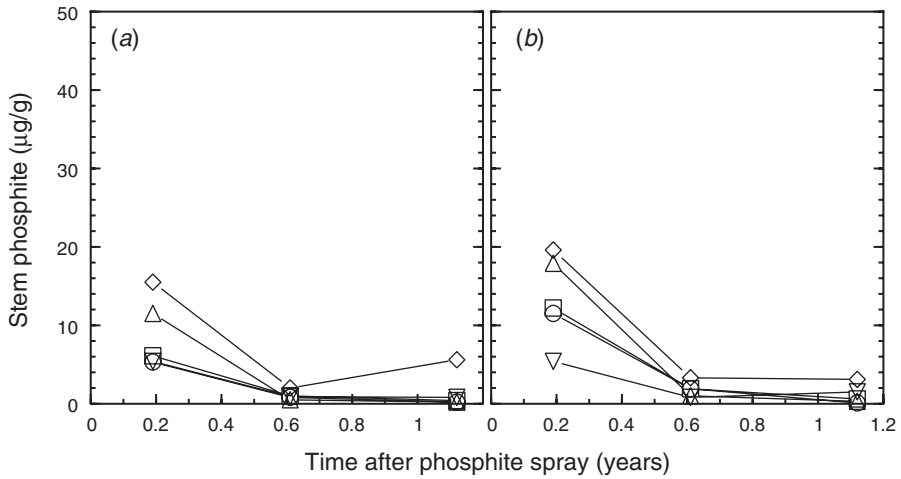

Fig. 6. Change in mean phosphite concentrations in stems of *Lambertia inermis* var. *inermis* with year after low-volume spray of (*a*) 24 and (*b*) 48 kg of phosphite/ha in 5 sites in the Esperance Plains bioregion of the South-West Botanical Province of Western Australia. Esperance Plains bioregion sites are by major soil group: Grey Deep Sand Duplex - DS1 (△) and DS2 (▽); Grey Shallow Sand Duplex - SS (○); Brown Loam - BL (□) and Red Loam - RL (◇). Significant ($P \leq 0.05$) linear relationships between variables: (*a*) 24 kg of phosphite/ha - BL: log Stem phosphite = –0.690 − 1.494 log Years after phosphite spray ($R^2 = 0.99$). (*b*) 48 kg of phosphite/ha) - DS1: log Stem phosphite = –0.975 − 2.294 log Years after phosphite spray ($R^2 = 0.99$); BL: log Stem phosphite = –0.251 − 1.674 log Years after phosphite spray ($R^2 = 0.99$).

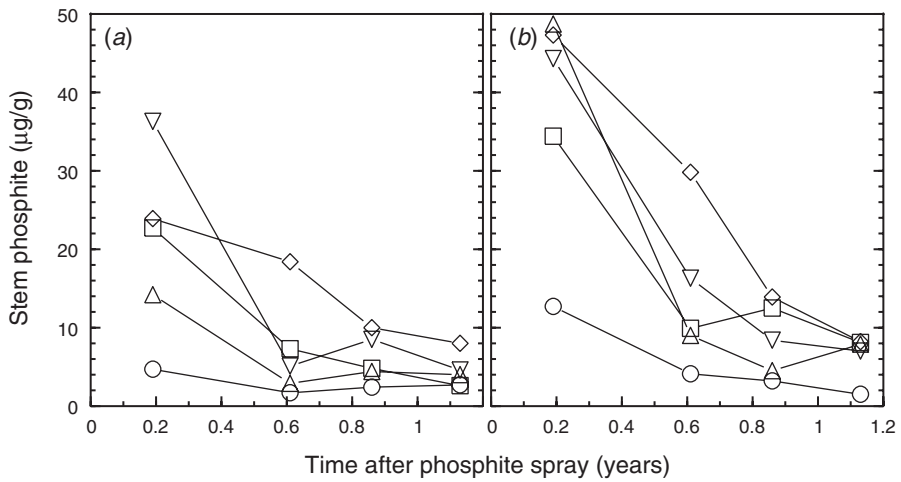

Fig. 7. Change in mean phosphite concentrations in stems of *Banksia grandis* with year after low-volume spray of (*a*) 24 and (*b*) 48 kg of phosphite/ha in 5 sites in Jarrah Forest bioregion of the South-West Botanical Province of Western Australia. Jarrah Forest bioregion sites by major Havel vegetation type: P (○); S1 (△); S2 (▽); T (□) and Q (◇). Significant ($P \leq 0.05$) linear relationships between variables: (*a*) 24 kg of phosphite/ha - T: Stem phosphite concentration = 3.042 − 11.550 log Years after phosphite spray ($R^2 = 0.98$). (*b*) 48 kg of phosphite/ha - P: Stem phosphite = 1.925 − 6.301 log Years after phosphite spray ($R^2 = 0.98$); S1: Stem phosphite = 3.696 − 25.314 log Years after phosphite spray ($R^2 = 0.90$); S2: Stem phosphite = 7.026 − 21.943 log Years after phosphite spray ($R^2 = 0.98$); T: Stem phosphite = 8.107 − 14.856 log Years after phosphite spray ($R^2 = 0.90$); Q: Stem phosphite = 12.896 − 21.798 log Years after phosphite spray ($R^2 = 0.94$).

There was a significant negative linear relationship between the logarithm of years after spray and the logarithm of phosphite concentrations in stems for BL site sprayed with 24 kg phosphite/ha and DS1 and BL sprayed with 48 kg phosphite/ha (Fig. 6). The decline of phosphite concentrations with time was consistently highest in site DS1, and BL tended to have a lower value (Fig. 6). While site DS1 had the fastest rates of decline of phosphite with time, the duplicate DS2 site conversely had the slowest rates of decline (Fig. 6).

Phosphite concentrations in *B. grandis* stems (Fig. 7) did not decline to the low levels occurring in *L. inermis* var. *inermis* stems (Fig. 6). By half a year after low-volume spray, phosphite

concentrations in *B. grandis* stems for all sites and spray rates were 14–76% of original concentrations (Fig. 7). Concentrations for the T and Q sites continued to decline up to 0.8 years after spraying. By 1.1 years after spray, phosphite concentrations had levelled off at concentrations 11–57% of original concentrations (Fig. 7).

There was a significant negative linear relationship between logarithm of year-after-spray and phosphite concentrations in stems for the T type site sprayed with 24 kg phosphite/ha and stems in all sites sprayed with 48 kg phosphite/ha (Fig. 7). The decline of phosphite concentrations with time was least for the P type site, but more varied for the other sites (Fig. 7). There were no consistent differences in decline in phosphite concentrations between duplicate type S sites.

Phosphite concentrations and colonisation

There was a significant positive linear relationship between phosphite concentrations and percentage inhibition of *P. cinnamomi* in *L. inermis* var. *inermis* stems for all Esperance Plains bioregion sites except BL (Fig. 8*a*). The rate of change of percentage inhibition of colonisation with increasing phosphite concentrations was greatest for DS2 and least for RL. The other sites were intermediate between the DS2 and RL. For the duplicate DS sites, the slope for DS2 was ≈ twice that for DS1.

There was a significant positive linear relationship between phosphite concentrations and percentage inhibition of *P. cinnamomi* in *B. grandis* stems for all Jarrah Forest bioregion sites except S1 (Fig. 8*b*). As in the case for Esperance Plains bioregion sites, the slope for the change of percentage inhibition with increasing phosphite concentrations was greatest for infertile sandy Havel type P site and least for the red loam type Q site (Fig. 8*b*). For the duplicate S sites, the linear relationship was significant for S2, but not for S1.

Correlations between variables

Significant correlations between variables for Esperance Plains bioregion and Jarrah Forest bioregion sites can be grouped into relationships between soil moisture or leaf phosphorus and soil attributes and the relationships affecting pathogen colonisation (Table 6). For Esperance Plains bioregion sites, soil moisture and leaf phosphorus tended to be less in infertile sites indicated by the significant negative relationship with coarse sand, and the positive relationships with fine sand and soil nutrients.

For Jarrah Forest bioregion sites, soil moisture tended to be greatest when environmental conditions were cool and wet as indicated by the significant negative relationship with temperature and the positive relationship with total rainfall and number of rain days. Leaf phosphorus tended to be less in infertile sites indicated by the significant negative relationship between bare ground and coarse sand, and its positive relationships with fine sand and soil nutrients.

For both bioregions the greatest colonisation by *P. cinnamomi* occurred in warm conditions, indicated by positive relationships with temperature and negative relationships with number of rain days. Also for both bioregions, least phosphite

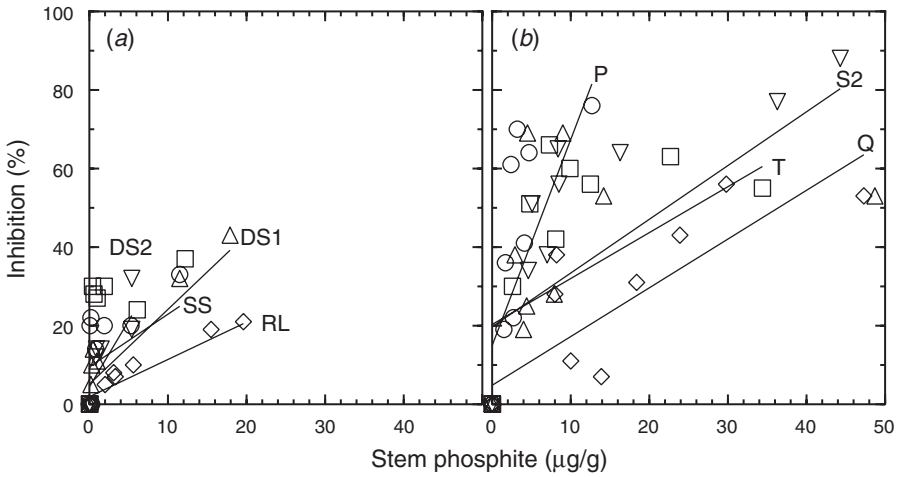

Fig. 8. Change in mean percentage inhibition of *Phytophthora cinnamomi* colonisation with mean phosphite concentrations in stems of either (*a*) *Lambertia inermis* var. *inermis* or (*b*) in stems of *Banksia grandis* after low-volume spray of 24 and 48 kg of phosphite/ha in 5 sites in the Esperance Plains or Jarrah Forest bioregions, respectively. (*a*) Esperance Plains bioregion sites by major soil group: Grey Deep Sand Duplex - DS1 (△) and DS2 (▽); Grey Shallow Sand Duplex - SS (○); Brown Loam - BL (□) and Red Loam - RL (◇). (*b*) Jarrah Forest bioregion sites by major Havel vegetation type: P (○); S1(△) and S2 (▽); T (□) and Q (◇). Lines indicate significant ($P \leq 0.05$) linear relationships between variables: (*a*) Esperance Plains bioregion (*L. inermis* var. *inermis*) - DS1: % inhibition = 5.084 + 2.191 Stem phosphite ($R^2 = 0.90$); DS2: % inhibition = 4.410 + 4.177 Stem phosphite ($R^2 = 0.74$); SS: % inhibition = 9.468 + 2.163 Stem phosphite ($R^2 = 0.49$); RL: % inhibition = 1.929 + 1.045 Stem phosphite ($R^2 = 0.94$). (*b*) Jarrah Forest bioregion (*B. grandis*) - P: % inhibition = 14.883 + 6.417 Stem phosphite ($R^2 = 0.58$); S2: % inhibition = 19.669 + 1.813 Stem phosphite ($R^2 = 0.67$); T: % inhibition = 20.283 + 1.760 Stem phosphite ($R^2 = 0.45$); Q: % inhibition = 4.828 + 1.343 Stem phosphite ($R^2 = 0.76$).

Table 6. Significant linear correlation coefficients ($P \leq 0.05$) for linear relationships between plant, lesion and site attributes for lesion development in stems of either *Lambertia inermis* var. *inermis* or *Banksia grandis* not sprayed or sprayed with 24 or 48 kg phosphite/ha and inoculated with *Phytophthora cinnamomi* and assessed at 3 times in 5 sites in the Esperance Plains bioregion or assessed at 4 times in 5 sites in the Jarrah Forest bioregion of the South-West Botanical Province of Western Australia

Bioregion (host)	Variable[A]	Variable[A]								
		MOIST	PLEAF	PHOS24	PHOS48	RATE0	RATE24	RATE48	INH24	INH48
Esperance Plains (*Lambertia inermis* var. *inermis*)	MOIST	1								
	TOTRAIN									
	RAINDAY				0.51	–0.57	–0.56	–0.59		0.66
	MINTEMP					0.56	0.51	0.55		
	MAXTEMP			–0.57	–0.65	0.84	0.72	0.70		
	CSAND	–0.71	–0.72							
	FSAND	0.72	0.75							
	CLAY								0.60	
	N	0.75								
	P	0.82								
	K	0.61								
	OC	0.76								
	BAREGRN	–0.70								
Jarrah Forest (*Banksia grandis*)	MOIST	1								
	TOTRAIN	0.74				–0.58	–0.51			
	RAINDAY	0.72		0.60	0.68	–0.74	–0.78	–0.63		
	MINTEMP	–0.85		–0.48	–0.52	0.64	0.65	0.43		
	MAXTEMP	–0.83			–0.47	0.60	0.60			
	CSAND		–0.68							
	FSAND		0.50							
	CLAY		0.59							
	N		0.81							
	P		0.92							
	K		0.80							
	OC		0.88							
	BAREGRN		–0.66							

[A]MOIST = % moisture in surface soil; PLEAF = phosphorus (μg/g) in new leaves of controls not sprayed with phosphite; PHOS24 = stem phosphite (μg/g) in plants sprayed with 24 kg of phosphite/ha; PHOS48 = stem phosphite (μg/g) in plants sprayed with 48 kg of phosphite/ha; RATE0, RATE24 and RATE48 = rate of colonisation (mm/day) of *P. cinnamomi* in stems sprayed with 0, 24 and 48 kg of phosphite/ha respectively; INH24 and INH48 = % inhibition of colonisation in stems sprayed with 24 and 48 kg of phosphite/ha respectively, compared with controls with no phosphite; TOTRAIN = total rain in mm during the period from inoculation to harvest; RAINDAY = number of rain days during the period from inoculation to harvest; MINTEMP = mean minimum temperature (°C) during the period from inoculation to harvest; MAXTEMP = mean maximum temperature during the period from inoculation to harvest; CSAND = % coarse sand in surface soil; FSAND = % fine sand in surface soil; CLAY = % clay in surface soil; N = % nitrogen in surface soil; P = phosphorus (μg/g) in surface soil; K = potassium (μg/g) in surface soil; OC = % organic carbon in surface soil; BAREGRN = % bare ground.

concentrations occurred in warm conditions, indicated by negative relationships with temperature. There was no significant correlation between soil nutrient variables and phosphite concentration, or inhibition of colonisation.

Discussion

Site

This is the first investigation to examine the effects of soil nutrient status on the effectiveness of low-volume spray of phosphite for the control of *P. cinnamomi*. As phosphate is a competitive inhibitor of phosphite uptake *in vitro* (Barchietto *et al*. 1988) and *in planta* (Smillie *et al*. 1989; Carswell *et al*. 1996), we hypothesised that soil phosphorus status may affect phosphite effectiveness in different communities. However, site was found to have a minimal influence on phosphite effectiveness in the two species of Proteaceae tested, despite relatively large differences in soil phosphate and other nutrients between sites, and the significant linear relationship between soil and leaf phosphorus observed for Jarrah Forest bioregion sites. Site factors influence plant physiological functions. Significant relationships occur between tissue nutrient concentration and soil nutrient levels for South-West Botanical Province of Western Australia flora and soils (Foulds 1993). However, this study suggests that the phosphate status of south-west Australian soils had minimal effect on phosphate-phosphite-*P. cinnamomi* interactions within the plant tissue of the two plant species tested. Ouimette and Coffey (1990) also found that uptake of phosphite by leaf disks of sugar beet and castor bean was not affected by external phosphate concentration.

The ancient, leached soils of south-western Australia are nutrient impoverished compared with world standards (Lamont 1995), and the Proteaceae have the lowest nutrient concentrations of 60 families tested (Foulds 1993). Thus, an alternative hypothesis may be that phosphate levels in the sites and plant species used in this study may be so low that they are below a threshold which must be exceeded before phosphate tissue levels interfere with phosphite effectiveness. Whether site has little

influence on phosphite effectiveness in taxa of genera other than Proteaceae will require further testing.

The lack of consistent differences observed between sites and correlation between soil variables, inhibition of colonisation and stem phosphite concentration suggests that site factors other than soil nutrient status may also have a minor effect on lesion development and phosphite effectiveness. However this should not discount the importance of examining the influence of other factors on phosphite responses - as factors such as light intensity, soil moisture and plant species composition will vary between sites; depending on climate, aspect, topographical position and soil age and profile characteristics. Plant death from drought was observed in site DS1 on the Esperance Plains bioregion and the P and Q site type in northern Jarrah Forest bioregion. The significant negative correlation obtained between Esperance Plains bioregion site attributes and surface soil moisture suggests that drought stress in the DS1 site was probably associated with infertile course sands. The DS1 site is more inland than the other Esperance Plains bioregion sites, and rainfall affecting soil water storage months before inoculation may also have been an important factor affecting plant water status. The P and Q sites had relatively shallow soil over impervious clay, so drought stress was probably due to rapid utilisation of soil water storage following below-average winter rains. Drought stress affected translocation of phosphite in *Xanthorrhoea preissii*, with translocation to the roots occurring in non-stressed plants in late winter but not in drought stressed plants in summer (Pilbeam *et al*. 2000). Furthermore, stem phosphite concentration was negatively correlated to temperature. The influence of drought stress on plant response to phosphite needs to be determined.

It was not unexpected that varying inoculation times during the course of the year was a major factor affecting rate of lesion development of *P. cinnamomi*, as temperature (Shearer *et al*. 1987*b*) and the moisture status of tissue being invaded (Tippett *et al*. 1987) are major determinants of pathogen growth rate in susceptible hosts of south-western Australia. Warm moist conditions favour rapid growth rates of *P. cinnamomi* in invaded tissue (Shearer and Tippett 1989; Shearer and Smith 2000). In this study, greatest colonisation by *P. cinnamomi* occurred in warm conditions as indicated by the positive correlation between these conditions and temperature, coupled with its negative correlation to rainfall. Low colonisation of *B. grandis* stems in summer was probably related to low tissue moisture status inhibiting *P. cinnamomi* growth rates (Tippett *et al*. 1987). Despite the considerable seasonal effect of temperature and moisture on *P. cinnamomi* growth rates in invaded tissue (Shearer *et al*. 1988; Tippett *et al*. 1989), phosphite spray in autumn and spring had minor influence on effectiveness of the fungicide (Tynan *et al*. 2001).

Phosphite concentrations in stems differed significantly and consistently between sites, in contrast to the effect of phosphite on *P. cinnamomi* colonisation which did not differ consistently or significantly between sites. Site fertility influenced phosphite concentration in both Esperance Plains bioregion and Jarrah Forest bioregion sites, with greater concentration in stems of plants in relatively fertile loam sites, and least in relatively infertile sandy sites. As phosphate is a competitive inhibitor of phosphite uptake *in vitro* (Barchietto *et al*.1988) and *in planta* (Smillie *et al*.1989; Carswell *et al*. 1996), it was hypothesised that soil phosphorus status may affect phosphite uptake and persistence in different communities. As phosphorus levels were 4 to 7 times greater in the loam than the sandy soil, greater inhibition of phosphite uptake by phosphate would be expected to occur in the relative fertile loam soils. That the reverse occurred supports previous conclusions for south-west Australian soils; that soil phosphate status had minimal effect on phosphate-phosphite-*P. cinnamomi* interactions within the plant tissue of the two plant species tested. However, the reasons for greater uptake of phosphite by plants in loam rather than sandy sites requires further investigation.

Site also affected the persistence of phosphite, but differences between sites were not consistent. Decline in stem phosphite concentrations in Esperance Plains bioregion sites tended to be greater for DS1 and least for DS2, both infertile sandy sites. In Jarrah Forest bioregion sites, this decline was smallest in infertile sandy site P whilst the fertile loam site Q had the most decline. Site influences on phosphite concentration in stems contrasts with previous conclusions that site had minimal effect on the effectiveness of phosphite for the control of *P. cinnamomi*. Phosphite has a complex mixed mode of action in sprayed plants (Smillie *et al*. 1989; Guest and Grant 1991) and low concentration in tissues stimulate host defence mechanisms (Afek and Sztejnberg 1989; Jackson *et al*. 2000). Thus, while phosphite uptake will depend more directly on the plant's physiological condition as mediated by environment, the influence of the fungicide on inhibition of fungal development is more indirectly determined through the stimulation of the host defence mechanisms. Even though the way site may influence phosphite uptake and persistence is unclear, the differences in phosphite uptake and persistence between sites observed still supports the conclusion that soil nutrient status had minimal effect on phosphate-*P. cinnamomi* interactions within the plant tissue of the two plant species tested.

Plant response

Fundamental differences in plant response to low-volume phosphite spray were observed between the two species of Proteaceae tested. Differences in phosphite uptake and persistence between south-west Australian native plant species has been reported previously (Komorek *et al*. 1997; Pilbeam *et al*. 2000; Barrett 2001; Tynan *et al*. 2001; Wilkinson *et al*. 2001; Shearer *et al*. 2007*a*). Phosphite was relatively ineffective in controlling *P. cinnamomi* colonisation in *L. inermis* var. *inermis* in association with poor uptake and persistence of the fungicide. In contrast low-volume phosphite spray was highly effective in controlling *P. cinnamomi* colonisation in *B. grandis*; phosphite uptake was 2–8 times greater, and persistence at least twice longer than that for *L. inermis* var. *inermis*. Relative differences between *L. inermis* var. *inermis* and *B. grandis* were not due to differences between the Esperance Plains bioregion and northern Jarrah Forest bioregion isolates and environments. Testing both plant species with the Esperance Plains bioregion isolate in the same site confirmed that phosphate controlled colonisation of *P. cinnamomi* in *B. grandis* but not *L. inermis* var. *inermis* (B.L. Shearer and C.E. Crane, unpubl. data). The response of *B. grandis* to low-volume phosphite spray was similar to that

found for high-volume spray (Tynan *et al*. 2001; Wilkinson *et al*. 2001; Shearer *et al*. 2006; Shearer and Fairman 2007*a*, 2007*b*) and we would consider it to be a phosphite-responsive species. In contrast, we would consider *L. inermis* var. *inermis* to be a phosphite-non-responsive species. The ineffectiveness of low-volume phosphite in *L. inermis* var. *inermis* is similar to the response of rare and endangered *L. echinata* subsp. *occidentalis*, where no protection from *P. cinnamomi* was obtained, even at high *in planta* phosphite concentration (R Smith, pers. comm.). However, *L. inermis* var. *inermis* may differ from *L. multiflora*, as Tynan *et al*. (2001) obtained effective control of *P. cinnamomi* in this plant species following high-volume sprays of phosphite. Inter-specific differences in phosphite effectiveness within *Lambertia* need to be determined. A high priority is the development of a database of plant species' responses to phosphite, in order to identify plant species in which phosphite effectively controls *P. cinnamomi*.

Phosphorus concentrations in leaves of *L. inermis* var. *inermis* were ≈ 4 times greater than in leaves of *B. grandis.* Requiring further investigation is whether the greater concentration of phosphorus in *L. inermis* var. *inermis* was sufficient to inhibit phosphite uptake and persistence in this species.

Heavy leaf fall was noted below both sprayed and non-sprayed *L. inermis* var. *inermis* plants. Slow transfer of phosphite from leaves and loss from leaf shed may have resulted in low phosphite concentrations being recorded in stems of *L. inermis* var. *inermis*. Phosphite uptake and persistence in horticultural crops is affected by source-sink relationships involving translocation of the fungicide (Whiley *et al*. 1995) and dilution and loss of the chemical through plant growth, fruiting, and shedding of leaves and roots (Ouimette and Coffey 1990; Whiley *et al*. 1995). A better understanding of differences in uptake and persistence of phosphite in native plant species requires time-course studies of phosphite uptake, distribution and loss budgets in the plant parts of different plant species in a range of environments.

That concentration of phosphite in stems increased with dosage is in agreement with previous observations (Komorek *et al*. 1997; Pilbeam *et al*. 2000; Barrett 2001; Tynan *et al*. 2001). Possibly the relatively low concentrations of phosphite in *L. inermis* var. *inermis* following low-volume spray of phosphite could be increased by changing the method of application. In this study, plants were sprayed once with a low-volume spray of phosphite, but current prescriptions use two low-volume sprays of 12–24 kg of phosphite/ha applied ≈ 6 week apart. Komorek *et al*.(1997) have shown that two low-volume sprays significantly increase tissue concentration of phosphite. The use of different application methods to increase tissue phosphite levels will require further investigation.

Significant positive linear relationships between stem phosphite concentration and percentage inhibition of *P. cinnamomi* colonisation were obtained for both low-responsive *L. inermis* var. *inermis* and high-responsive *B. grandis* in all but two sites. While the relationship between phosphite concentration and percentage inhibition of *P. cinnamomi* colonisation was mirrored in effective colonisation control by low-volume phosphite sprays in highly responsive *B. grandis*, the relationship did not reflect effective control in *L. inermis* var. *inermis*. This suggests that tissue phosphite concentrations in *L. inermis* var. *inermis* are below a threshold needed for effective control. Effective control was achieved in *B. grandis* in site type P, where stem phosphite concentrations were similar to that obtained in *L. inermis* var. *inermis*-suggesting that *B. grandis* has a lower effective threshold than *L. inermis* var. *inermis*. These observations can be extended to a general hypothesis that plant species differ in species-specific phosphite tissue thresholds that must be exceeded before effective control of *P. cinnamomi* can be achieved. Plant species having a low-response to phosphite have a higher effective threshold than those that have a high-response to phosphite. These thresholds need to be determined for different plant species and environments.

El-Hamalawi *et al.* (1995) found a significant negative linear relationship between phosphite concentration in fresh tissue and logarithm of canker size for *P. citricola* in avocado. They suggest that bark tissue phosphite concentration of 21 μg/g fresh weight or higher was required for 'complete disease prevention' in the *P. citricola*-avocado pathosystem. A significant linear correlation between phosphite concentration and lesion development for *B. hookeriana* in a glasshouse environment (Wilkinson *et al*. 2001) is the only previous report for native flora of south-western Australia. Currently there is insufficient information to indicate threshold phosphite tissue concentrations that will result in effective control of *P. cinnamomi* following phosphite spray in different plant species and environments in the long term. Time-course studies are required of phosphite tissue concentrations in relation to colonisation inhibition.

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References

Afek U, Sztejnberg A (1989) Effects of Fosetyl-Al and phosphorus acid on scoparone, a phytoalexin associated with resistance of citrus to *Phytophthora citrophthora. Phytopathology* **79**, 736–739. doi: 10.1094/Phyto-79-736

Barchietto T, Saindrenan P, Bompeix G (1988) Characterization of phosphite uptake in two *Phytophthora* spp. and its inhibition by phosphate. *Archives of Microbiology* **151**, 54–58. doi: 10.1007/BF00444669

Barrett SR (2001) Phytotoxic effects of phosphite in native plant communities in southern Western Australia. Ph.D. Thesis, Murdoch University.

Barrett S (2003) Monitoring of aerial phosphite applications for the control of *Phytophthora cinnamomi* in the Albany district. In '*Phytophthora* in forests and natural ecosystems. 2nd International IUFRO Working Party 7.02.09 Meeting, Albany, W. Australia'. (Eds JA McComb, GE StJ Hardy, IC Tommerup) pp. 132–137. (Murdoch University Print, Murdoch)

Barrett SR, Shearer BL, Hardy GEStJ (2002) Root and shoot development in *Corymbia calophylla* and *Banksia brownii* after the application of the fungicide phosphite. *Australian Journal of Botany* **50**, 155–161. doi: 10.1071/BT01018

Boesewinkel HJ (1976) Storage of fungal cultures in water. *Transactions of the British Mycological Society* **66**, 183–185.

Carswell C, Grant BR, Theodorou ME, Harris J, Niere JO, Plaxton WC (1996) The fungicide phosphonate disrupts the phosphate-starvation response in *Brassica nigra* seedlings. *Plant Physiology* **110**, 105–110.

Cochrane JA, Crawford AD, Monks LT (2007) The significance of *ex situ* seed conservation to reintroduction of threatened plants. *Australian Journal of Botany* **55**, 356–361. doi: 10.1071/BT06173

Day PR (1965) Particle fraction and particle-size analysis. In 'Methods of soil analysis. Part 1'. Agronomy Monograph 9 (Ed. CA Black) pp. 545–567. (ASA and SSSA: Madison Wisconsin)

El-Hamalawi ZA, Menge JA, Adams CJ (1995) Methods of Fosetyl-Al application and phosphonate levels in avocado tissue needed to control stem canker caused by *Phytophthora citricola. Plant Disease* **79**, 770–778.

Foulds W (1993) Nutrient concentrations of foliage and soil in south-western Australia. *The New Phytologist* **125**, 529–546. doi: 10.1111/j.1469-8137.1993.tb03901.x

Guest D, Grant B (1991) The complex action of phosphonates as antifungal agents. *Biological Reviews of the Cambridge Philosophical Society* **66**, 159–187. doi: 10.1111/j.1469-185X.1991.tb01139.x

Havel JJ (1975) Site-vegetation mapping in the northern jarrah forest (Darling Range). Definition of site-vegetation types. Bulletin 86. (Forests Department: Perth)

Jackson TJ, Burgess T, Colquhoun I, Hardy GEStJ (2000) Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi. Plant Pathology* **49**, 147–154. doi: 10.1046/j.1365-3059.2000.00422.x

Kirby KN (1993) 'Advanced data analysis with SYSTAT.'(Van Nostrad Reinhold: New York).

Komorek B, Shearer BL, Smith B, Fairman RG (1997) The control of *Phytophthora* in native plant communities. In 'Control of *Phytophthora* and *Diplodina* Canker in Western Australia. Final Report to the Threatened Species and Communities Biodiversity Group, Environment Australia'. (Ed. D Murray) pp. 1–59. (Department of Conservation and Land Management: Perth)

Lamont BB (1995) Mineral nutrient relations in Mediterranean regions of California, Chile, and Australia. In 'Ecology and biogeography of Mediterranean ecosystems in Chile, California, and Australia.' (Eds MT Kalin Arroyo, PH Zedler, MD Fox) pp. 211–235. (Springer-Verlag: New York)

McKenzie HA, Wallace HS (1954) The Kjeldahl determination of nitrogen: a critical study of digestion conditions - temperature, catalyst and oxidising agent. *Australian Journal of Chemistry* **7**, 55–70.

Mead R (1988) 'The design of experiments: statistical principles for practical application'. (Cambridge University Press: Cambridge).

Monks L, Coates D (2002) The translocation of two critically endangered *Acacia* species. *Conservation Science Western Australia* **4**, 54–61.

Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**, 403–405. doi: 10.1034/j.1600-0706.2003.12010.x

Murphy J, Riley T (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**, 31–36. doi: 10.1016/S0003-2670(00)88444-5

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858. doi: 10.1038/35002501

Ouimette DG, Coffey MD (1990) Symplastic entry and phloem translocation of phosphonate. *Pesticide Biochemistry and Physiology* **38**, 18–25. doi: 10.1016/0048-3575(90)90143-P

Pilbeam RA, Colquhoun IJ, Shearer B, Hardy GEStJ (2000) Phosphite concentration: its effect on phytotoxicity symptoms and colonisation by *Phytophthora cinnamomi* in three understorey species in *Eucalyptus marginata* forest. *Australasian Plant Pathology* **29**, 86–95. doi: 10.1071/AP00016

Piper CS (1942) 'Soil and plant analysis' (University of Adelaide: Adelaide)

Schoknecht N (2002) Soil groups of Western Australia. A simple guide to the main soils of Western Australia. Resource Management Technical Report 246. (Department of Agriculture: South Perth)

Shea SR, Gillen KJ, Leppard WI (1980) Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in soil in diseased, freely drained *Eucalyptus marginata* Sm sites in northern jarrah forest of south-western Australia. *Protection Ecology* **2**, 135–156.

Shearer BL, Crane CE (2003) The influence of soil from a topographic gradient in the Fitzgerald River National Park on mortality of *Banksia baxteri* following infection by *Phytophthora cinnamomi*. In '*Phytophthora* in Forests and Natural Ecosystems. 2nd International IUFRO Working Party 7.02.09 Meeting, Albany, W. Australia'. (Eds JA McComb, GE StJ Hardy, IC Tommerup) pp. 268. (Murdoch University Print, Murdoch)

Shearer BL, Fairman RG (2007*a*) A stem injection of phosphite protects *Banksia* species and *Eucalyptus marginata* from *Phytophthora cinnamomi* for at least four years. *Australasian Plant Pathology* **36**, 78–86. doi: 10.1071/AP06085

Shearer BL, Fairman RG (2007*b*) Application of phosphite in a high-volume foliar spray delays and reduces the rate of mortality of four *Banksia* species infected with *Phytophthora cinnamomi. Australasian Plant Pathology* **36**, 358–368. doi: 10.1071/AP07033

Shearer BL, Smith IW (2000). Diseases of eucalypts caused by soilborne species of *Phytophthora* and *Pythium*. In 'Diseases and Pathogens of Eucalypts'. (Eds PJ Keane, GA Kile, FD Podger, BN Brown) pp. 259–291. (CSIRO Publishing: Canberra)

Shearer BL, Tippett JT (1989) Jarrah dieback: The Dynamics and Management of *Phytophthora cinnamomi* in the Jarrah (*Eucalyptus marginata*) Forest of South-western Australia. Department of Conservation and Land Management Research Bulletin 3.

Shearer BL, Crane CE, Cochrane A (2004*a*) Quantification of the susceptibility of the native flora of the South-West Botanical Province, Western Australia, to *Phytophthora cinnamomi. Australian Journal of Botany* **52**, 435–443. doi: 10.1071/BT03131

Shearer BL, Crane CE, Fairman RG (2004*b*) Phosphite reduces disease extension of a *Phytophthora cinnamomi* front in *Banksia* woodland, even after fire. *Australasian Plant Pathology* **33**, 249–254. doi: 10.1071/AP04002

Shearer BL, Fairman RG, Grant MJ (2006) Effective concentration of phosphite in controlling *Phytophthora cinnamomi* following stem injection of *Banksia* species and *Eucalyptus marginata. Forest Pathology* **36**, 119–135. doi: 10.1111/j.1439-0329.2006.00440.x

Shearer BL, Michaelsen BJ, Somerford PJ (1988) Effects of isolate and time of inoculation on invasion of secondary phloem of *Eucalyptus* spp. and *Banksia grandis* by *Phytophthora* spp. *Plant Disease* **72**, 121–126. doi: 10.1094/PD-72-0121

Shearer BL, Michaelsen BJ, Warren HJ (1987*a*) Comparative behaviour of *Phytophthora* species in the secondary phloem of stems and excised roots of *Banksia grandis* and *Eucalyptus marginata. Australian Journal of Botany* **35**, 103–110. doi: 10.1071/BT9870103

Shearer BL, Shea SR, Deegan PM (1987*b*) Temperature-growth relationships of *Phytophthora cinnamomi* in the secondary phloem of roots of *Banksia grandis* and *Eucalyptus marginata. Phytopathology* **77**, 661–665. doi: 10.1094/Phyto-77-661

Shearer BL, Crane CE, Barrett S, Cochrane A (2007*a*) *Phytophthora cinnamomi* invasion, a major threatening process to conservation of flora diversity in the South-West Botanical Province of Western Australia. *Australian Journal of Botany* **55**, 225–238. doi: 10.1071/BT06019

Shearer BL, Crane CE, Barrett S, Cochrane A (2007*b*) Assessment of threatened flora susceptibility to *Phytophthora cinnamomi* by analysis of disease progress curves in shadehouse and natural environments. *Australasian Plant Pathology* **36**, 609–620. doi: 10.1071/AP07074

Smillie R, Grant BR, Guest D (1989) The mode of action of phosphite: evidence for both direct and indirect modes of action on three *Phytophthora* spp. in plants. *Phytopathology* **79**, 921–926. doi: 10.1094/Phyto-79-921

Tippett JT, Crombie DS, Hill TC (1987) Effect of phloem water relations on the growth of *Phytophthora cinnamomi* in *Eucalyptus marginata. Phytopathology* **77**, 246–250. doi: 10.1094/Phyto-77-246

Tippett JT, Hill TC, Shearer BL (1985) Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi. Australian Journal of Botany* **33**, 409–418. doi: 10.1071/BT9850409

Tippett JT, McGrath JF, Hill TC (1989) Site and seasonal effects on susceptibility of *Eucalyptus marginata* to *Phytophthora cinnamomi. Australian Journal of Botany* **37**, 481–490. doi: 10.1071/BT9890481

Tsao PH, Guy SO (1977) Inhibition of *Mortierella* and *Pythium* in *Phytophthora*-isolation medium containing hymexazol. *Phytopathology* **67**, 796–801.

Tynan KM, Wilkinson CJ, Holmes JM, Dell B, Colquhoun IJ, McComb JA, Hardy GEStJ (2001) The long-term ability of phosphite to control *Phytophthora cinnamomi* in two native plant communities of Western Australia. *Australian Journal of Botany* **49**, 761–770. doi: 10.1071/BT00062

Whiley AW, Hargreaves PA, Pegg KG, Doogan VJ, Ruddle LJ, Saranah JB, Langdon PW (1995) Changing sink strengths influence translocation of phosphonate in avocado (*Persea americana* Mill.) trees. *Australian Journal of Agricultural Research* **46**, 1079–1090. doi: 10.1071/AR9951079

Wilkinson CJ, Holmes JM, Tynan KM, Colquhoun IJ, McComb JA, Hardy GEStJ, Dell B (2001) Ability of phosphite applied in a glasshouse trial to control *Phytophthora cinnamomi* in five plant species native to Western Australia. *Australasian Plant Pathology* **30**, 343–351. doi: 10.1071/AP01055

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