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Phosphite concentration: its effect on phytotoxicity symptoms and colonisation by *Phytophthora cinnamomi* in three understorey species of *Eucalyptus marginata* forest

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Abstract

Pre-treatment of plants with foliar sprays of 0.2, 0.5 and 2% phosphite restricted colonisation by *Phytophthora cinnamomi* in inoculated stems of *Adenanthos barbiger* and *Daviesia decurrens*, and led to a reduction in the isolation of *P. cinnamomi* from these stems in comparison with unsprayed plants. In plants treated with 2% phosphite, *P. cinnamomi* was not isolated from *D. decurrens* but was isolated from 22% of the stems of *A. barbiger*. In *Xanthorrhoea preissii*, colonisation by, and isolation of, *P. cinnamomi* from inoculated roots was not significantly affected by pre-treatment of the foliage with 0.2, 0.5 and 2% phosphite. Very low concentrations of phosphite were detected in the roots of *X. preissii* (maximum mean of 2.2 μg/g dry weight), in comparison with the phosphite concentrations measured in the foliage of *A. barbiger* and *D. decurrens* plants treated with phosphite (maximum means of 80 and 871 μg/g dry weight, respectively). Treatment with 0.2% phosphite resulted in minimal phytotoxicity in each of the three species, whereas treatment with 2% phosphite led to the development of severe phytotoxicity symptoms. This study indicates that phosphite has potential for the management of *P. cinnamomi* in native plant communities.

Introduction

Infestation by the soilborne plant pathogen, *Phytophthora cinnamomi* Rands, is a major threat to the ecology and conservation of many plant communities in Australia (Shearer and Tippett 1989). *P. cinnamomi* is responsible for disease and death in overstorey and understorey components of these plant communities. The first indication of the presence of *P. cinnamomi* in *Eucalyptus marginata* Donn. Ex Smith forest is the death of susceptible understorey species. Highly susceptible species, such as *Adenanthos barbiger* Lindley (family Proteaceae), *Daviesia decurrens* Meissner (family Fabaceae) and *Xanthorrhoeapreissii* Endl. (family Xanthorrhoeaceae) serve as good indicators of infestation (Shearer and Dillon 1995).

The main objectives for the management of *P. cinnamomi* in Western Australian native plant communities are to minimise both the introduction and spread of the pathogen, and the intensification of the disease (Conservation and Land Management 1994). Recently, trials have been conducted to examine the effectiveness of stem injection and foliar spraying of the systemic fungicide phosphite. Phosphite is currently used to protect rare and endangered plant species and rare native ecosystems (Conservation and Land Management 1994).

Although phosphite is considered to have low phytotoxicity (Guest and Grant 1991), phytotoxicity symptoms have developed in a wide range of plant species after its application. These include horticultural crops such as mandarins (Walker 1989), almond and cherry trees (Wicks and Hall 1988; 1990) and carrots (Walker 199 1). The lowest concentration of phosphite at which leaf bum has been reported is 0.4% phosphite, where a foliar application on mandarins (Walker 1989) and azaleas (de Boer and Greenhalgh 1990) led to necrosis of the leaf tips. In a study of native plants in Victoria, 25% of the species tested developed phytotoxicity symptoms after treatment with 0.6% phosphite (Aberton *et al.* 1999). There is anecdotal evidence that understorey plants of native communities in the southwest of Western Australia are also susceptible to the phytotoxic effects of phosphite. In preliminary studies conducted by the Department of Conservation and Land Management (CALM), the foliar application of 1% phosphite led to the death of plants in the genus *Petrophile,* the development of leaf necrosis in X. *preissii,* and reduced growth in *Banksia coccinea R.* Br. (Shearer, unpublished results).

The aims of this study were to examine the effect of phosphite concentration on phytotoxicity to *A. barbiger, D, decurrens* and X. *preissii,* and colonisation by *P. cinnamomi* in stems of *A. barbiger* and *D. decurrens,* and roots of X. *preissii.*

Methods

The trials were conducted in *E. marginata* forest at Jarrahdale, Western Australia (1 l6"7'E, 32'22's). This area of Western Australia has a Mediterranean climate, experiencing hot dry summers and cool wet winters, with an average annual rainfall of 1300 mm. The site was an 'S' type according to Havel's vegetation type (Have1 1975) and was in a disease centre. E. *marginata* was the major overstorey species, with a middle storey of *Banksia grandis* Willd. and understorey dominated by *A. barbiger, Bossiaea ornata* (Lindley) Benth., *D. decurrens* and *X. preissii.* The site was gently sloping, with a southwesterly aspect. The soil was orange lateritic gravel with loamy sand.

Experiment 1: Phytotoxicity trial

Experimental design The independent variable was treatment with 0, 0.2, 0.5 and 2% phosphite (equivalent to 0, 0.2, 0.5 and 2% phosphite/L), while the dependent variables were the phytotoxicity symptoms, occurrence of new growth and flower production. There were 20 replicate plants of *A. barbiger, D. decurrens* and *X. preissii* per treatment, selected from an area approximately 50 x 50 m and assigned treatments randomly. The height range of the selected plants was from 250 to 700 cm in *A. barbiger,* 290 to 980 cm in *D. decurrens* and 7 10 to 1690 cm in *X. preissii.*

Spray application Plants received a single foliar application of phosphite to run-off. Phosphite solutions were prepared from Fosject 200 (Unitec Group Pty Ltd), which contains 200g phosphite/L, present as mono-di potassium phosphite. All treatments contained 0.25% Synertrol oil (Organic Crop Protectants Pty Ltd), which was mixed with the phosphite prior to dilution with water. The prepared solutions were agitated constantly while spraying with a backpack sprayer. *A. barbiger* and *D. decurrens* were sprayed in late March and X *preissii* in

early April (maximum ambient temperatures 31°C and 24°C, respectively). The total rainfall in Jarrahdale from the beginning of December until the day of spraying in March was 5 mm, with a further 5.2 mm rainfall before *X. preissii* was sprayed.

Monitoring A. barbiger and *D. decurrens* were assessed for phytotoxicity symptoms every second day for a week, and *X. preissii* was assessed every third day for 2 weeks. Symptoms were rated according to a phytoxixity rating system developed from a preliminary trial (Table 1).

The recovery of plants was monitored monthly for 6 months. The occurrence of new growth in all three species was recorded cumulatively, on an absent/present basis. The flowering of *A, barbiger* and *D. decurrens,* and the production of seedpods in *D. decurrens* subsequent to flowering, was recorded monthly in the last 3 months of monitoring, also on an absent/present basis. The plants from which the inoculated stems had been harvested (Experiment 2) were removed from the data set for the frequency of new growth and production of flowers and seedpods.

Phosphite analysis Five weeks after spraying with phosphite, leaves of *A. barbiger* and phyllodes of D. *decurrens* were harvested for phosphite analysis, with two replicate samples from each of seven plants per phosphite treatment. Leaves/phyllodes of similar maturity were collected from the stems that had been inoculated with *P. cinnamomi* 31 days prior to harvesting (Experiment 2). The samples were washed in warm water with a small amount of phosphate-free detergent (Colgate-Palmolive, Australia) and rinsed in warm water before drying at 37°C for 7 days. They were subsequently ground into a fine powder using an electric grinding machine.

For each replicate, a sample of 0.1 g was taken, to which 1.9 mL sulphuric acid (0.1 N) was added.

Table 1 Rating systems developed to assess phytotoxicity in *Adenanthos barbiger, Daviesia decurrens* and *Xanthorrhoea preissii* after the foliar application of phosphite

Rating	Symptoms in hosts		
	A. barbiger	*D. decurrens*	*X. preissii*
0	No leaf burn	No phyllode burn	No leaf burn
1	Leaf burn restricted to old growth	Burn restricted to margins/tips of phyllodes	Leaf burn restricted to new growth
2	Leaf burn on old growth and affecting less than 50% of the new growth	Phyllode burn not restricted to margins or tips, affecting less than 50% of the plant	Leaf burn on new and old growth, affecting less than 50% of the plant
3	Leaf burn on old growth and affecting greater than 50% of the new growth	Severe phyllode burn, affecting greater than 50% of the plant	Severe leaf burn, affecting greater than 50% of the plant

The samples were shaken and left overnight, then centrifuged at 8000 rpm for 10 min before 250 µL of the supernatant was added to 1 mL methanol. Phosphite standards were prepared to cover the range of 1 to 1000 µg/mL phosphite.

An excess of diazomethane was added to each sample and the dimethyl phosphite content was analysed using gas chromatography (with a P-sensitive column) and a flame photometric detector. With such small samples, the limit of detection was 1 µg/g dry weight tissue.

Statistical analysis For the results of new growth and flower production in *A. barbiger,* and flower and seedpod production in D. *decurrens,* the 95% confidence interval was calculated and used to determine if there was a significant difference between phosphite treatments.

The *in planta* phosphite concentrations were normalised by a $\log_{10} (x+1)$ transformation prior to determining the Spearman's rank order correlation between phosphite concentration and phytotoxicity rating. For statistical purposes, values of zero were used where phosphite could not be detected.

Experiment 2: Phosphite concentration and *P. cinnamomi* colonisation of *A. barbiger, D. decurrens* and *X. preissii*

Experimental design The independent variable was pre-treatment with phosphite. Plants from Experiment 1 were used, with seven plants per species selected randomly from each of the four phosphite treatments (0, 0.2, 0.5 and 2% phosphite). In *A. barbiger* and *D. decurrens,* six stems per plant were inoculated with *P. cinnamomi* 7 days after the foliage had been sprayed with phosphite, plus one uninoculated stem per plant. *X preissii* roots were inoculated with *P. cinnamomi* 30 days after the foliage had been sprayed with phosphite. Where there were sufficient roots available, seven roots per plant were inoculated with colonised Miracloth discs, plus one uninoculated root per plant. The dependent variables were the length of colonisation, and isolation of *P. cinnamomi* from the inoculated stems/roots.

Inoculum material The isolate used (Murdoch Culture collection, MP94-48) was originally isolated from *E. marginata* and has previously been found to be highly virulent on *E. marginata* (Hüberli 1995). The isolate was maintained on half-strength potato-dextrose agar (½PDA) plates [containing potato-dextrose agar (PDA) and bacteriological agar (BA)].

Colonised Miracloth (Calbiochem, United States of America) discs were prepared by placing agar plugs colonised with MP94-48 around sterile 5 mm diameter Miracloth discs on ½PDA plates. Colonised discs were used 7 days later, and sterile Miracloth discs were used in uninoculated treatments.

Inoculation A. barbiger was inoculated by cutting the periderm through to the cambium on each stem with a razor blade. With *D. decurrens,* the phyllodes were pulled away slightly to expose the stem tissue. One Miracloth disc was placed in each opening, and the inoculation point was then sealed with Parafilm (American National Can, United States of America) and wrapped with plastic flagging tape.

The roots of X *preissii* were prepared *5* weeks prior to inoculation. A trench approximately 0.5 m from each plant was dug with a mechanical backhoe. The trenches were between 0.3 and 0.6 m deep. Soil around the plant base was removed with a hand trowel to expose the roots. The soil was replaced with vermiculite, to which approximately 8 L water per plant was added. Black plastic sheeting was placed around the vermiculite which was then covered with soil. In early May, roots between 8 and 40 cm long with diameters ranging from 1 to 4 mm, were gently exposed and inoculated with either colonised or sterile Miracloth discs. The roots were inoculated as described above for *A. barbiger.* After inoculation, the vermiculite, plastic sheeting and soil were replaced.

Water relations Since the plants had experienced a very *dry* summer and autumn, the water potentials of *A. barbiger* and *D. decurrens* were measured to assess whether drought stress was likely to be limiting colonisation by the pathogen in the host tissue. Measurements were made on leaves/phyllodes and stems that were situated away from the inoculated stems.

The water potential of the seven replicate plants o f A. barbiger and *D. decurrens* that had been treated with 0% phosphite was determined using the pressure chamber method of Scholander et al. (1965). Pre-dawn and midday measurements were made on a sunny day in early May (maximum ambient temperature 20°C). One stem (approximately 10 cm long) was excised from each plant with a razor blade, wrapped in plastic and trans-ported as quickly as possible to the pressure chamber.

The water potential of *X. preissii* plants treated with 0% phosphite was measured 1 day before the root inoculations. The method used was similar to that described above, except that leaves rather than stems were used. Pre-dawn and midday measurements were made on the seven replicate plants prepared for inoculation, plus four *X. preissii* plants with undisturbed soil to determine if the root excavation process had affected plant water potential.

Harvesting *A. barbiger* and *D. decurrens* stems were harvested 31 days after inoculation. Recovered Miracloth discs were plated onto P_5ARH, a Phytophthora-selective agar (containing pimaricin, ampicillin, rifampicin, hymexacol, pentachloronitrobenzene, PDA and BA) (O'Gara 1997), before 1 cm stem segments were cut up to 8 cm above and below the inoculation point. The segments were split in half longitudinally and then plated sequentially onto the selective agar. Plates were incubated in the dark at 25°C and examined every second day for 6 days for the presence o f *P. cinnamomi*. The extent of colonisation was deter-mined by recording the segments from which *P. cinnamomi* was isolated. The isolation of *P. cinnamomi* was determined by calculating the percentage of stems from which *P. cinnamomi* was isolated.

The roots of *X. preissii* were harvested 23 days after inoculation. The Miracloth discs were recovered and plated onto the P_5ARH selective agar before the roots were surface sterilised in 70% ethanol for 15 sec and allowed to dry. Six 1 cm segments from above and below the inoculation point on each root were split in half longitudinally then plated sequentially. Plates were incubated and examined as described above.

Phosphite analysis The leaves and phyllodes from the inoculated stems of *A. barbiger* and *D. decurrens* were analysed for phosphite in early May, as described in Experiment 1.

With *X. preissii*, phosphite analysis was conducted on the root segments that remained after plating. Some of the roots were surrounded by a bark layer, which was removed before the roots were washed, dried, ground and analysed as described in Experiment 1.

Statistical analysis The treatment effects on length of colonisation and isolation o f *P. cinnamomi* were analysed by one-way ANOVA after $\log_{10}(x + 1)$ transformation and angular transformation, respectively. Where examination of residuals indicated that these were heteroscedastic, the significant result from the ANOVA was confirmed by the non-parametric Kruskal-Wallis test, following the procedure recommended by Fry (1993). Pairwise comparisons were made using the Tukey HSD test.

Results

Experiment 1: Phytotoxicity trial

Phytotoxicity symptoms Phytotoxicity symptoms developed in A. barbiger, *D. decurrens* and *X. preissii* at phosphite concentrations of 0.2, 0.5 and 2% within 14 days of phosphite application. The severity of symptoms increased with higher phosphite concentrations (Table 2). No phytotoxicity symptoms developed from the sprays which contained Synertrol and water only.

The phosphite concentration in the leaves of *A. barbiger* and phyllodes of *D. decurrens* increased with an increase in the concentration of phosphite applied to the foliage (Table 2). The phyllodes of *D. decurrens* contained higher concentrations of phosphite than the leaves of *A. barbiger,* particularly in the 2% phosphite treatment, where the concentration of phosphite in *D. decurrens* was ten times higher than that detected in *A. barbiger* leaves.

There was a significant ($P<0.0l$) positive correlation between the transformed phosphite concentration in *A. barbiger* and the phytotoxicity rating *(* $r = 0.780$). In *D. decurrens,* the correlation between the two was significant ($P<0.01$) and strongly positive *(*$r = 0.929$).

Recovery from phytotoxicity, new shoot growth and flowering In each of the three species, the necrotic leaves/phyllodes were retained on the majority of plants for the duration of the trial. The visible damage to the foliage was irreversible. Recovery was apparent in the form of new growth.

Over the 6 months of monitoring *A. barbiger,* resprouting from beneath the soil surface and from stems was evident in plants for each phosphite treatment and the nil treatment. Plants treated with 0% phosphite did not produce new growth until 6 months after treatment, whereas 17% of the treated plants had produced new growth in the 3 months after spray application. Plants treated with 0.5 and 2% phosphite produced significantly ($P<0.05$) more growth during the first 3 months of monitoring than the untreated plants (Figure 1a). By the sixth month, the frequency of new growth in plants treated with 0.2 and 2% phosphite was

significantly ($P<0.05$) greater than in those treated with 0% phosphite (Figure lb). The new growth was not affected by phytotoxicity, but was frequently damaged by insects.

Six months after spraying, there was no significant ($P>0.05$) difference between phosphite treatments in the number of plants flowering.

In *D. decurrens* new growth in the form of resprouting from the basal part of the stems occurred in only two individuals. These had been treated with 0.2 and 2% phosphite. All new growth was healthy. After treatment with 2% phosphite, there were significantly ($P<0.05$) less plants producing flowers and seedpods in comparison with the 0, 0.2 and 0.5% treatments (Figures 2a and b).

Three months after the application of phosphite, all the X *preissii* individuals affected by phytotoxicity had healthy new growth emerging from the centre of the stem. No flowering occurred in *X. preissii.*

Table 2 Phytotoxicity rating, in planta phosphite concentration in leaves/phyllodes/roots, and recovery/isolation of *Phytophthora cinnamomi* from Miracloth discs, stems of *Adenanthos barbiger* and *Daviesia decurrens* and roots of *Xanthorrhoea preissii* after the foliar application of phosphite and inoculation with *Phytophthora cinnamomi*

Plant species	Phosphite concentration applied (%)	Phytotoxicity rating (mean, n=20)	*In planta* phosphite concentration (µg/g dry wt)[A]	Isolation of *P. cinnamomi* from stems/roots (%)	Recovery of *P. cinnamomi* from Miracloth discs (%)
A. barbiger	0	0	0 a	88 ±7a[B]	79 ±5a[B]
	0.2	0.5	4 ±1b[C]	55 ±8a	62 ±9a
	0.5	1	7 ±1c	26 ±8b	64 ±7a
	2.0	2	80 ±10d	22 ±7b	45 ±11a
D. decurrens	0	0	0 a	33 ±16a	72 ±12a
	0.2	0.5	18 ±3b	2 ±2b	73 ±6a
	0.5	2	107 ±22c	2 ±2b	71 ±7a
	2.0	3	871 ±42d	0 b	50 ±9a
X. preissii	0	0	1.7±0.2a	49 ±12ab	24 ±9a
	0.2	0.05	0.5±0.2a	60 ±7ab	47 ±10a
	0.5	1	2.2±0.7a	36 ±9a	52 ±13a
	2.0	2	2.1±1.3a	78 ±9b	33 ±11a

[A]For *Adenanthos barbiger* leaves, *Daviesia decurrens* phyllodes and *Xanthorrhoea preissii* roots.
[B]Untransformed means ± s.e of mean; within column for each species, means followed by the same letter are not significantly different ($P>0.05$), based on statistical analyses of data after angular transformation (n=7).
[C]Untransformed means ± s.e of mean; within column for each species, means followed by the same letter are not significantly different ($P>0.05$), based on statistical analyses of data after $\log_{10}(x+1)$ transforma-tion (n=7).

Experiment 2: Phosphite concentration and *P. cinnamomi* colonisation of *A. barbiger, D. decurrens* and *X.* preissii

Water relations The average pre-dawn and mid-day water potentials were -0.49 and-0.86 MPa in *A. barbiger,* -0.62 and -1.63 MPa in *D. decurrens* and -0.88 and -1.67 MPa in *X. preissii,* respectively. The average water potential in the X. *preissii* plants not prepared for inoculation was slightly less negative, though not significantly (P>0.05).

Colonisation of inoculated stems/roots by P. cinnamomi There was a significant (P<0.02) treatment effect in the extent of colonisation by *P. cinnamomi* in the stems of *A. barbiger* and *D. decurrens.* In *A. barbiger,* plants sprayed with 0.2% phosphite had 84% less colonisation than those treated with 0% phosphite. Treatments with 0.5 and 2% phosphite resulted in further reductions (Figure 3a), though these were not significantly (P>0.05) less than in the 0.2% treatment. In *D. decurrens,* there was 99-100% less colonisation in the plants treated with 0.2, 0.5 and 2% phosphite than in the 0% phosphite treatment (Figure 3b).

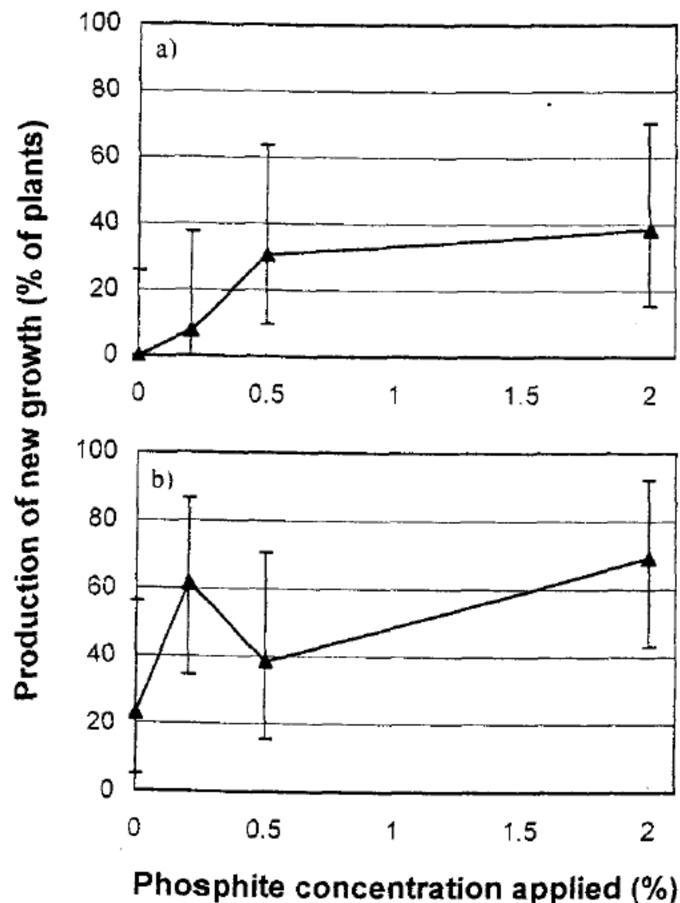

Figure 1. The effect of phosphite concentration on the production of new growth in *Adenanthos barbiger* a) 3 months and b) 6 months after foliar treatment. Bars represent the 95% confidence interval for each value (n= 13).

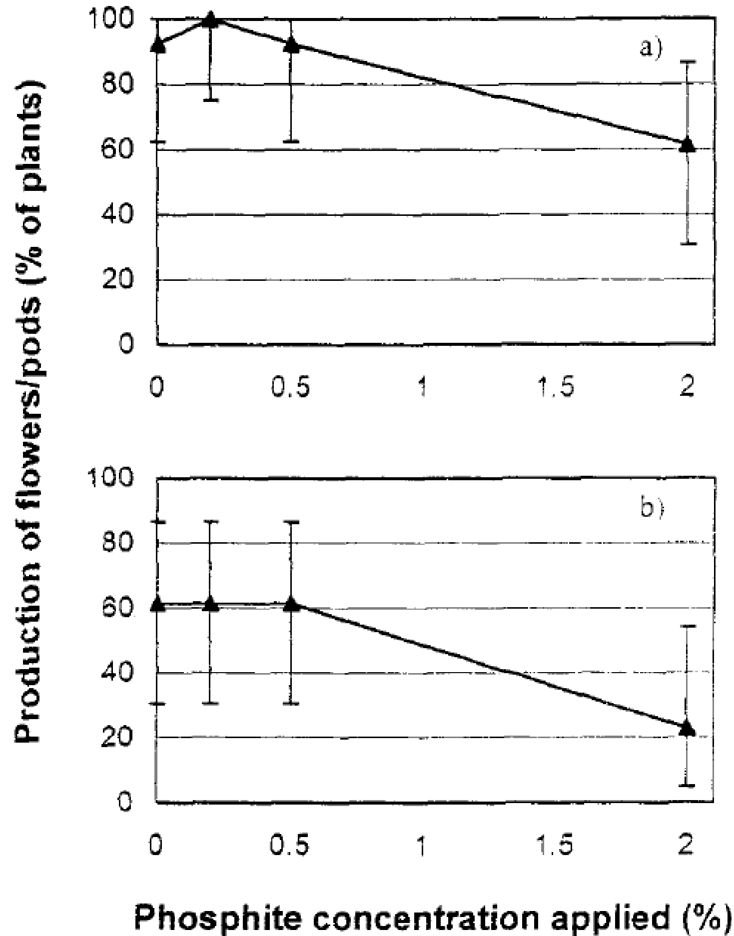

Figure 2. The effect of phosphite concentration on the percentage of *Daviesia decurrens* plants producing a) flowers and b) seedpods 3 to 6 months after foliar treatment. Bars represent the 95% confidence interval for each value (n= 13).

P. cinnamomi was isolated from 88.1 and 54.8% of the stems of *A. barbiger* plants treated with 0 and 0.2% phosphite, respectively, and there was no significant (P>0.05) difference between these treatments. There was a significantly (P<0.005) lower isolation of *P. cinnamomi* from the plants treated with 0.5 and 2% phosphite than in the 0% treatment (Table 2).

The isolation off *P. cinnamomi* from the inoculated stems of D. *decurrens* was 32.8% in the plants sprayed with 0% phosphite. The isolation of *P. cinnamomi* from the plants treated with 0.2, 0.5 and 2% phosphite was significantly (P<0.05) lower than in the 0% treatment (Table 2).

The extent of colonisation by *P. cinnamomi* in inoculated roots of *X. preissii* was similar in all phosphite treatments (Figure 3c), with no significant (P>0.05) differences between the 0% phosphite treatment and 0.2, 0.5 or 2% treatments. The extent of colonisation reached the maximum that could be measured (up to 6 cm away from the inoculation point) in 2.5, 2.4, 10 and 14% of the roots of plants treated with 0, 0.2, 0.5 and 2% phosphite, respectively.

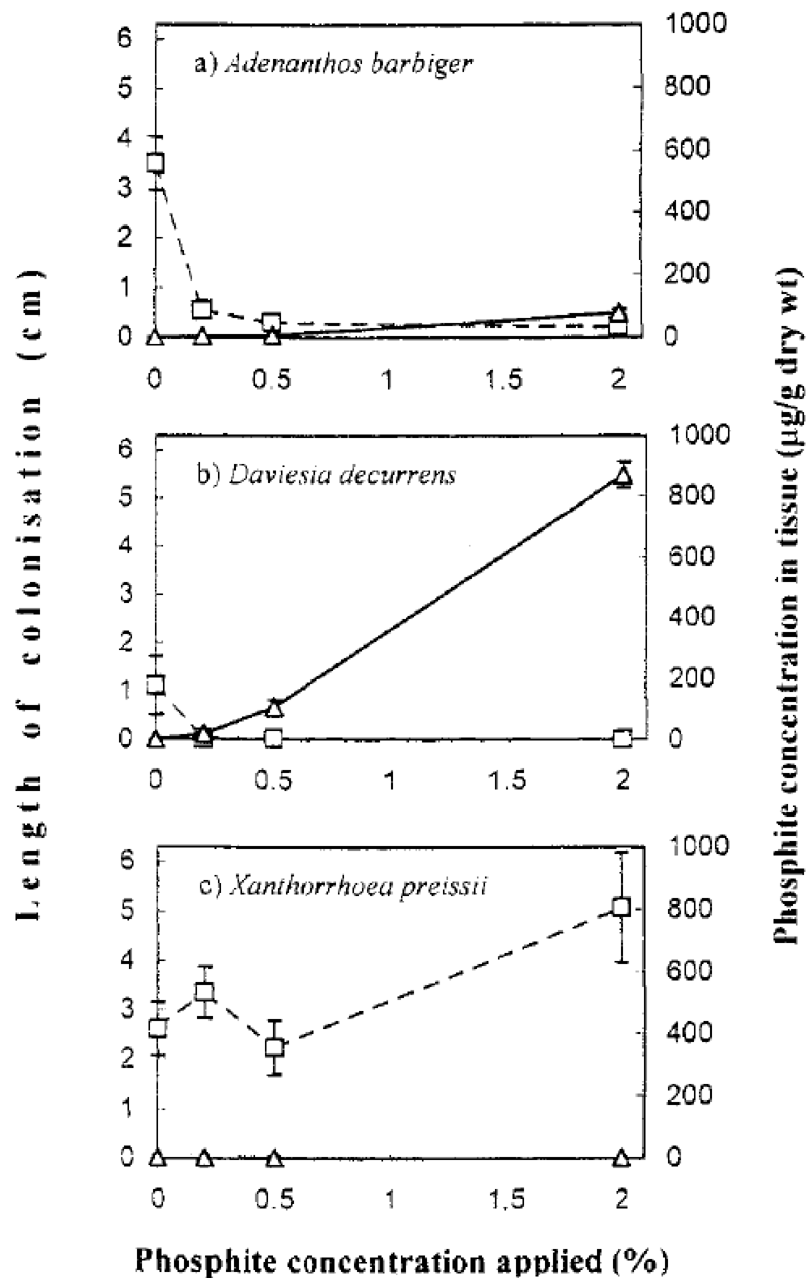

Figure 3. Length of colonisation (□) of *Phytophthora cinnamomi* in a) stems of *Adenanthos barbiger,* b) stems of *Daviesia decurrens* and c) roots of *Xanthorrhoea preissii,* and phosphite concentration (△) in a) leaves of *Adenanthos barbiger,* b) phyllodes of *Daviesia decurrens* and c) roots *of Xanthorrhoea preissii* in plants treated with phosphite and inoculated with *Phytophthora cinnamomi.* Bars represent the standard errors of the mean (n=7).

P. cinnamomi was isolated from 49% of inoculated roots of *X. preissii* plants treated with 0% phosphite, and the isolation of *P. cinnamomi* from plants treated with 0.2, 0.5 or 2% phosphite was not significantly ($P<0.05$) different from this (Table 2). The maximum isolation of *P. cinnamomi* (77.8%) was from the plants treated with 2% phosphite, and this was significantly ($P<0.05$) higher than the isolation of *P. cinnamomi* from plants treated with 0.5% phosphite. No *P. cinnamomi* was isolated from stems or roots inoculated with sterile Miracloth discs.

Recovery of P. cinnamomi from Miracloth discs In *A. barbiger* and *D. decurrens,* the recovery of *P. cinnamomi* from the Miracloth discs was lower in the plants receiving the 2% phosphite treatment than in those treated with 0, 0.2 and 0.5% phosphite, although not significantly (Table 2). There were fewer recoveries of *P. cinnamomi* from the Miracloth discs in *X. preissii* than in the other species in all phosphite treatments (Table 2). The recovery from Miracloth discs was not consistent with the isolation of *P. cinnamomi* from inoculated stems or roots.

In planta phosphite concentration and P. cinnamomi colonisation of stems/roots In *A. barbiger,* the length of colonisation and isolation of *P. cinnamomi* from the stems declined as the phosphite concentration in the leaves increased (Figure 3a and Table 2). The dosage effect was more apparent between the 0.2 and 0.5% treatments, where small differences in the phosphite concentration in planta led to differences in the extent of colonisation and isolation. In comparison, the length of colonisation and isolation of *P. cinnamomi* were similar in *A. barbiger* plants treated with 0.5 and 2% phosphite, despite an eleven-fold difference in phosphite concentration in the leaves.

In *D. decurrens*, phosphite concentrations of 18 and 107 µg/g dry weight of phyllodes in plants treated with 0.2 and 0.5% phosphite, respectively, corresponded with restricted colonisation and a low isolation of *P. cinnamomi* from the inoculated stems (Figure 3b and Table 2). *P. cinnamomi* was not isolated from any *D. decurrens* plants treated with 2% phosphite, where the average phosphite concentration in planta was 871 µg/g dry weight of phyllodes.

The phosphite concentrations in the roots of *X. preissii* were low and not significantly ($P>0.05$) different between phosphite treatments (Table 2). There was no apparent relationship between the extent of colonisation or isolation of *P. cinnamomi* and phosphite concentration in the roots (Figure 3c and Table 2).

Phytotoxicity and colonisation of stemshoots by P. cinnamomi In *A. barbiger* and *D. decurrens,* increasingly higher phytotoxicity ratings corresponded with a decline in the extent of colonisation and isolation of *P. cinnamomi* from the inoculated stems (Table 2 and Figure 3a and b).

In *X. preissii,* a low level of phytotoxicity after treatment with 0.5% phosphite corresponded with the least extent of colonisation and isolation of *P. cinnamomi* from the inoculated roots (Figure 3c and Table 2). In contrast, the most extensive colonisation and greatest isolation of *P. cinnamomi* were from the roots of plants treated with 2% phosphite, a highly phytotoxic

concentration. The two plants with the most severe phytotoxicity symptoms had the most extensive colonisation in their roots.

Discussion

This study demonstrated that the foliar application of 0.2, 0.5 and 2% phosphite was effective in the restriction of colonisation by *P. cinnamomi* in stems of *A. barbiger* and *D. decurrens,* but not in the roots of X. *preissii.*

Although treatment with 2% phosphite was slightly more effective than 0.5% phosphite in reducing stem colonisation and isolation of *P. cinnamomi,* it resulted in severe phytotoxicity symptoms. Necrosis developed in *A. barbiger, D. decurrens* and *X. preissii* at a phosphite concentration as low as 0.2%, which is below the recommended concentration of 0.3% for the control of *P. cinnamomi* in Australian native plants. This is in direct contrast with Guest and Grant (199l), who state that phosphite has low phytotoxicity. However, the observed phytotoxicity symptoms were never severe in the 0.2 or 0.5% phosphite treatments.

Necrosis occurred primarily at the margins or tips of leaves/phyllodes in each of the three species. Similar marginal and tip necrosis has been reported previously in a variety of plants in response to the application o f phosphite, including cocoa (Anderson and Guest 1990), azaleas (de Boer and Greenhalgh 1990), mandarins and carrots (Walker 1989; 1991) and almond and cherry trees (Wicks and Hall 1988; 1990).

The application of phosphite stimulated the production of new growth in *A. barbiger, D. decurrens* and *X. preissii,* particularly at the higher concentrations of phosphite and higher phytotoxicity ratings. It would appear that the damage caused by the phosphite initiated growth in buds present in the aerial and subterranean tissues. Such resprouting is considered to be an adaptation to stresses of many types, including fire (Gill 1981; Bell *et al.* 1993), drought and mechanical damage (Rundel 1981). Despite suffering extensive damage to the foliage after the application of 2% phosphite, the species treated in the present study possessed adaptations that enabled their recovery from the phytotoxic effects of phosphite. Nearly two-thirds of the species of plant communities in southwestern Western Australia are resprouters (Bell *et al.* 1993). However, the remaining one third are obligate seeders, and the effect of phosphite on plants with this life history syndrome is not known. The observed effect of 2% phosphite on flowering and reproduction in *D. decurrens* indicates that high concentrations of phosphite may be detrimental to the long-term survival of plant species and their dependant fauna. The short- and long-term effects of phosphite on flowering and reproduction in native plants are currently being investigated.

The moderate phytotoxicity symptoms that developed after the application of 0.5% phosphite did not predispose *A. barbiger* or *D. decurrens* to more colonisation by *P. cinnamomi.* However, the effects of phytotoxicity on the susceptibility of treated plants to other biotic and abiotic factors are not known. Once plants are stressed, they become susceptible to opportunistic pathogens (Isaac 1992). Phytotoxicity should be avoided until there is a better understanding of its consequences. Further studies should investigate seasonal effects on the

phytotoxicity of phosphite to a range of species and determine the time at which maximum concentrations can be applied with minimal phytotoxicity.

Wound inoculation of stems has been found to be a useful and practical method for the assessment of the susceptibility of *E. marginata* to colonisation by *P. cinnamomi* (Tippett *et al.* 1985; Shearer *et al.* 1988). Stem inoculations of *A. barbiger* and *D. decurrens* demonstrated the potential for phosphite to restrict the growth of *P. cinnamomi* in susceptible native plants. The use of stems has previously been considered a conservative estimate of the effectiveness of phosphite, since comparisons of phosphite concentrations in shoots and roots have found greater concentrations of phosphite in the roots of *Banksia telmetia, Lambertia multiflora* (Komorek and Shearer 1997) and *Corymbia calophylla* (Fairbanks *et al.,* in press; Barrett, unpublished results). The phloem mobility of phosphite has also been reported in avocado plants (Ouimette and Coffey 1989) and citrus plants (Schutte *et al.* 1991).

In contrast to reports of the phloem mobility of phosphite, it would appear that phosphite was not translocated from the leaves to the roots of *X. preissii* when sprayed in autumn. However, in a subsequent spray of *X. preissii* with 2% phosphite in August (late winter), phosphite concentrations of 18 and 10 µg/g dry weight were detected in the roots 1 and 4 weeks after spraying, respectively (Pilbeam, unpublished results). In autumn, drought stress may have inhibited the uptake and/or translocation of phosphite in *X. preissii.* The water relations measured in *D. decurrens* and *X. preissii* were in the range of water stress that has been shown to restrict the growth of *P. cinnamomi* in *E. marginata* (Tippett *et al.* 1987). Studies on herbicides have shown reduced translocation in drought-stressed plants (Reynolds *et al.* 1988; Peregoy *et al.* 1990; Morrison *et al.* 1995). For an assessment of the effectiveness of a foliar application of phosphite in containing a root pathogen, the factors that influence phosphite uptake and distribution should be investigated before proceeding with root inoculations.

The results indicate that *P. cinnamomi* can be contained in plants treated with phosphite but may not be killed in the plant. It is generally accepted that phosphite does not eradicate the pathogen (Conservation and Land Management 1994), and hygiene practices are still required to minimise the spread of the pathogen. Although expensive and impractical as a routine treatment for extensive areas, phosphite can be used to protect rare communities and may be useful in the containment of spot infections. More research needs to be done to determine how to maximise the control of *P. cinnamomi* while minimising any harmful effects of phosphite on plant and animal communities.

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