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Effective concentration of phosphite in controlling *Phytophthora cinnamomi* following stem injection of *Banksia* species and *Eucalyptus marginata*

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Summary

The effect of phosphite concentration on lesion development by *Phytophthora cinnamomi* in stems and roots of *Banksia grandis* and *Eucalyptus marginata* and in stems of *Banksia coccinea* was assessed during a 4.3 year period after stem injection of phosphite. Lesion length 6 weeks after inoculation was significantly less in roots of *B. grandis* trees that had been stem injected with three concentrations of phosphite (50, 100 and 200 g phosphite/l) at two rates (1 and 2 ml/cm of stem circumference) compared with the not-injected control. With the exception of *B. grandis* trees injected with 50 g phosphite/l, lesion length for the high rate was not significantly different to the low rate. In roots of *E. marginata*, lesion development in response to phosphite was different to that in roots of *B. grandis*; lesion length in roots did not differ significantly between phosphite concentration and rate. Lesion length and girdling in stems of *B. grandis* and *E. marginata* was significantly less in those injected with phosphite than in not injected stems. One year after injection, callus tissue had contained lesions in stems injected with phosphite. By 4.3 years after injection of both hosts there was a steep significant negative linear relationship between phosphite concentration and either lesion length or girdling, with greatest lesion development in not injected stems and least in stems injected with 100 g phosphite/l. Recovery of *P. cinnamomi* from lesion margins 1 year after injection, was significantly less in trees injected with phosphite than in not injected trees. The amount of plant death reflected containment of lesion extension and girdling, and reduction of recovery of *P. cinnamomi* with phosphite concentration; 4.3 year after injection there was a steep significant negative linear relationship between phosphite concentration and percentage of plant death. In contrast to *B. grandis* and *E. marginata*, there was a U-shaped non-linear relationship between phosphite concentration and effectiveness of phosphite in controlling lesion extension and girdling in *B. coccinea*. Containment of lesion extension and girdling with time was greatest for *B. coccinea* stems injected with 25 g phosphite/l, least for stems not injected, and intermediate in stems injected with 50 and 100 g phosphite/l. As in *B. grandis* and *E. marginata*, containment of lesion extension and girdling in *B. coccinea* with phosphite concentration was reflected in the amount of plant death. The non-linear response to phosphite of some plant species indicated that injected concentration for *B. coccinea* should not exceed 50 g phosphite/l, whereas injected concentrations of up to 100 g phosphite/l could be recommended for *B. grandis*. Longevity of action of phosphite for 4–5 years in native plant species after one injection makes phosphite injection a practical control option for the control of *P. cinnamomi* disease front extension and the protection of threatened flora. Research into the effect of factors affecting longevity of action of phosphite would facilitate optimization of timing of injection.

1 Introduction

Disease caused by the introduced pathogen *Phytophthora cinnamomi* Rands infection significantly reduces floristic composition and structural complexity of vegetation in south-western Australia and is a major threat to the ecology and conservation of the

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susceptible plant communities of the region (Shearer et al. 2004a). Greatest impact of the pathogen occurs in the understorey of *Eucalyptus marginata* Donn ex Smith forest on laterites, and in *Banksia* woodlands associated with leached sands and laterites of the Northern and Southern Sandplains and the Swan Coastal Plain (Shearer 1990, 1994). Movement of infected soil by human activity has spread the pathogen throughout the region, and favourable climatic and soil profile conditions, together with a large number of susceptible hosts, ensure continued impact of *P. cinnamomi* on these communities (Shearer and Tippett 1989; Shearer 1994).

Many of the communities vulnerable to infection by *P. cinnamomi* contain rare flora or fauna and their conservation requires control of existing infestations and protection from introduction of the pathogen. Disease management has largely relied on mapping of the disease, and containment of the pathogen using hygienic procedures originally developed in *E. marginata* forest (Shearer and Tippett 1989). However, as hygienic procedures do not change the environment affecting the pathogen, they are mainly delaying tactics that do not alter the rate of disease development once established. There has therefore been a need to test other options, such as fungicides, that can alter the environment to disfavour the pathogen, and delay and reduce the rate of disease development (Shearer and Tippett 1989). However no safe, effective and cheap fungicides were suitable for the treatment of native communities until after the mid 1980's, when phosphite (Averbuch-Pochot and Durif 1996) was demonstrated to be effective in the control of *P. cinnamomi* in agriculture (Cohen and Coffey 1986; Guest and Grant 1991) and in native ecosystems using foliar application (Shearer and Fairman 1991, 1997; Aberton et al. 1999; Hardy et al. 2001). High sensitivity of *P. cinnamomi* to the fungicide, stimulation of host defence mechanisms, low cost in comparison with other fungicides, low toxicity to animals and truly systemic translocation in both the phloem and xylem (Cohen and Coffey 1986; Guest and Grant 1991) indicated that phosphite could be a promising control option for the protection of native communities of high conservation value from *P. cinnamomi* infestation.

Stem injection of phosphite has been effectively used in agriculture to protect avocado (Darvas et al. 1984; Pegg et al. 1987; El-Hamalawi et al. 1995), cocoa (Guest et al. 1994), citrus (Schutte et al. 1991), pome (Long et al. 1989), stone fruits (Wicks and Hall 1988) and chestnut (Lim 1993) against disease caused by *Phytophthora* species. However, stem injection of phosphite has not been tested in native communities and the effect of phosphite concentration in the injecting solution on lesion development over time has been little studied. Moreover, differences between agriculture and native community situations such as management practices, soil fertility, tree physiology and losses in harvested fruit and leaf fall, negate extrapolation of results from agriculture to native plant communities. This study aims to determine the effective concentration of stem-injected phosphite for long-term control of *P. cinnamomi* lesions in native plant communities.

2 Materials and methods

2.1 Experimental design

There were two experiments in which different phosphite concentrations were injected into *B. grandis* Wild. and *E. marginata* Donn. ex Smith. In a third experiment, different concentrations were injected into *B. coccinea* R. Br. For these three experiments there were single-tree replicates in a randomized block design. In a fourth experiment effectiveness of injected phosphite was also tested along *P. cinnamomi* disease fronts in *B. coccinea* communities.

Experiment 1. The objective of the experiment was to evaluate the effect of phosphite concentration on lesion development in roots of *B. grandis* and *E. marginata* assessed

Table 1. **Characteristics of sites where trees were injected with phosphite**

Characteristic	Site 1	Site 2	Site 3	Site 4
Community	*E. marginata* forest	*E. marginata* forest	*B. coccinea* woodland	*B. coccinea* woodland
Latitude (S)	32°41′ 53″	32°37′ 18″	34°58′ 32″	34°34′ 47″
Longitude (E)	116°03′ 33″	116°00′ 44″	117°59′ 20″	118°08′ 40″
Local topography	Convex mid-slope	Convex mid-slope	Mid-slope of old dune	Mid-slope of old dune
Aspect	West	North	North	North
Soil type	Laterite	Laterite	Sand	Sand
Soil at 0–10 cm				
Colour	Brown	Brown	Grey	Grey
Coarse sand (%)	59.5	45.4	73.2	53.9
Fine sand (%)	33.8	43.3	21.6	38.9
Silt (%)	3.1	4.8	1.2	2.8
Clay (%)	3.5	6.5	4.1	4.4
Nitrogen (%)	0.03	0.04	0.11	0.11
Phosphorus (μg/g)	17.1	31.0	18.4	12.0
Potassium (μg/g)	31.6	61.7	11.3	23.9
Organic carbon (%)	1.8	2.6	2.9	3.8
pH	5.2	5.4	5.0	4.7
Soil at 40–60 cm				
Colour	Brown	Brown	Grey-white	Grey-white
Coarse sand (%)	51.8	49.2	76.4	42.2
Fine sand (%)	42.4	40.5	23.0	56.1
Silt (%)	0.6	2.7	0.3	1.1
Clay (%)	5.2	7.6	0.2	0.6
Nitrogen (%)	0.05	0.08	0.02	0.02
Phosphorus (μg/g)	28.5	32.3	8.2	5.7
Potassium (μg/g)	26.8	71.4	<1.0	<1.0
Organic carbon (%)	0.3	0.5	0.2	0.8
pH	5.5	5.7	4.7	4.6

6 weeks after inoculation. At site 1 (Table 1) the roots of stems of 20–30 year old trees of *B. grandis* with circumference over bark of 29–52 cm [mean (±SEM) of 37.7 ± 0.4 cm] and 50–70 year old trees of *E. marginata* with circumference over bark of 49–112.5 cm (mean of 77.5 ± 0.8 cm) were exposed. Diameters of *B. grandis* and *E. marginata* roots were not significantly different ($p = 0.63$) Root diameter was 0.8–8.0 cm (mean of 2.2 ± 0.2 cm) for *B. grandis* and 0.8–5.2 cm (mean of 2.1 ± 0.2 cm) for *E. marginata*. Three weeks after the roots were inoculated with *P. cinnamomi*, the stems were not injected or injected with three concentrations of phosphite (50, 100 and 200 g phosphite/l) at two rates (1 or 2 ml/cm of stem circumference). The dependent variables of lesion length and girdling of roots by *P. cinnamomi*, isolation of the pathogen and crown health was assessed on 10 single tree replicates for each host-phosphite treatment combination, 6 weeks after inoculation.

Experiment 2. The objective of the experiment was to evaluate the effect of phosphite concentration on lesion development in stems of *B. grandis* and *E. marginata* assessed during a 4.3 year period after injection. At site 2 (Table 1), stems of 30 year old trees of *B. grandis* with circumference over bark of 20–42 cm (mean of 29.5 ± 0.3 cm) and 30–40 year old trees of *E. marginata* with circumference over bark of 21–50 cm (mean of 33.1 ± 0.4 cm) were not injected or injected with four concentrations of phosphite (12.5, 25, 50, and 100 g phosphite/l) at the rate of 1 ml/cm of stem circumference, 3 weeks after inoculation. Lesion length and girdling of stems by *P. cinnamomi*, isolation of the pathogen

and tree mortality were the dependent variables assessed at each sampling time. Sufficient trees were treated for a new set to be assessed at each sampling time. Ten single tree replicates for each host-phosphite treatment combination were assessed at 0.06, 0.12, 1, 2 and 4.3 years after injection.

Experiment 3. The objective of the experiment was to evaluate the effect of phosphite concentration on lesion development in stems of *B. coccinea* assessed during a 3.7 year period after injection. At site 3 (Table 1), stems of 20–30 year old trees of *B. coccinea* with circumference over bark of 19–64 cm (mean of 33.7 ± 0.8 cm) were not injected or injected with three concentrations of phosphite (25, 50, and 100 g phosphite/l) at the rate of 1 ml/cm stem circumference, 3 weeks after inoculation. Lesion length and girdling of stems by *P. cinnamomi*, isolation of the pathogen and tree mortality were the dependent variables assessed at each sampling time. Because of limited number of trees, the same trees were assessed at each sampling time. Ten single tree replicates for each phosphite treatment were assessed at 0.06, 0.12, 0.7, 1, 2 and 3.7 years after injection.

Experiment 4. The objective of the experiment was to evaluate the effect of phosphite concentration on mortality of *B. coccinea* growing along disease fronts. In early December, 1989, 20–30 year old trees with circumference over bark of 15–58 cm (mean of 31.1 ± 1.5 cm) along the disease front in site 4 (Table 1) were divided into 32 pairs of trees close to one another and of similar size. Within each pair of trees, the stem of one tree at random was injected with 100 g phosphite/l at the rate of 1 ml/cm stem circumference and the other tree was not injected. Mortality was monitored at various times over a 6 year period (Fig. 6a). In early June, 1992, 20–30 year old trees along the disease front in site 3 (Table 1) were divided into 19 groups of three trees close to one another and of similar size. Within each group, the stem of one tree at random was injected with 25 g phosphite/l, another injected with 50 g phosphite/l and the third tree not injected. Phosphite solutions were injected at the rate of 1 ml/cm stem circumference. Mortality was monitored approximately every 6 months over a 4.8 year period (Fig. 6b).

2.2 Sites

Details of the sites are given in Table 1. The trees used in experiments 1–3 were situated in healthy forest or woodland, downslope of *P. cinnamomi* disease fronts. In the forest sites, *B. grandis* trees were dispersed amongst the trees of *E. marginata* used in the study. Soil from each site was bulked, air dried and analysed using the following methods. Phosphorous content was determined colorimetrically using the method of Murphy and Riley (1962). The potassium content was determined using the same solution and a flame photometer. The organic carbon content was determined by the Walkley-Black method (Piper 1942). Soil nitrogen was determined by extracting the soil with 1 M 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. Percentages by weight of sand silt and clay of the fine fraction were determined by the pipette method (Day 1965).

2.3 Inoculation

In experiment 1, soil was removed from two roots per tree and each root inoculated 1.5 m from the stem. In experiments 2 and 3 the stems were inoculated 1.5 m above the soil surface. Roots and stems were wound-inoculated in summer with *P. cinnamomi* isolate SC72 (IMI 264384), using methods previously described (Shearer et al. 1987a,b, 1988). Isolate SC72 was chosen because it was used in previous studies (Shea et al. 1980; Tippett et al. 1983, 1985, 1987; Shearer et al. 1987a,b, 1988) and its pathogenicity was not statistically different from other *P. cinnamomi* isolates (Shearer et al. 1988). An agar disk

containing mycelium of SC72 was bound to a fresh cut in the phloem. Control stems were inoculated in a similar manner with sterile agar disks. In experiment 1, roots were inoculated in mid-January, 1988. In experiment 2 and 3, stems were inoculated in mid-March 1991 and the end of November 1991, respectively.

2.4 Injection

Three weeks after inoculation with *P. cinnamomi*, stems were injected with aqueous phosphite solutions made from 20% commercial formulation [Fosject-200, UIM Agro-chemicals (Australia) Pty Ltd (Rocklea, Queensland, Australia) containing 200 g $H_2(PO_3H)$/l present as the mono-di potassium phosphite, adjusted to pH 5.7–6.0]. Holes were drilled with a 6.5 mm bit into the sapwood, at equal distance around the stem and 0.5 m above the soil surface. The required volume of phosphite solution injected into each tree and the number of syringes per tree depended on tree size as measured by stem circumference. The canopies of the trees were too sparse to be an accurate measure of tree size. The volume was equally divided into at least two or more syringes per tree, with a maximum of 20 ml per syringe. The required volume of phosphite solution was sucked up into a catheter tip syringe and the air expelled. The nozzle was placed through a 10 mm hole in the centre of a 100 × 20 mm metal plate, to which was attached two 60-mm long and 6-mm outside diameter metal springs. The nozzle was inserted into the drilled hole by pushing and turning to give an airtight fit. While holding the syringe with one hand, the plunger was gently pulled back until back pressure was felt, indicating an airtight fit of the syringe. A constant positive pressure was applied to the plunger by fastening the ends of the two springs attached to the metal plate, into holes at the end of the plunger. The time for uptake of the solution varied from 2 to 10 min for a healthy *B. grandis* to up to 24 h for a large *E. marginata.* Within a year after injection the drilled holes had callused over and no secondary infection occurred.

2.5 Assessment

In experiment 1, crown density was determined 3 weeks after injection using the nine-point scale of Grimes (1987) which varied from a rating of nine for very dense crowns, to five for average and one for very sparse crowns. Two roots per tree were also harvested and transverse and longitudinal cuts were made through the point of inoculation with a band saw and the cut surfaces trimmed. Lesion length above and below the inoculation point was measured.

In experiments 2 and 3, lesion size was determined on the tree. The bark was carefully scraped from the lesion margins above and below and at each side of the inoculation point. Lesion length above and below the inoculation point was measured and girdling by the lesion at the point of inoculation was estimated. For experiments 1 and 2, the presence of *P. cinnamomi* was verified by plating 10 pieces of tissue at the lesion margin onto selective medium (Tsao and Guy 1977). In experiment 3 the same trees were assessed with time and in order to prevent repeated damage to the lesioned tissue, no samples were taken from the lesion margin.

2.6 Statistical analysis

Assumptions of normality were checked by plotting residuals (Kirby 1993). Length and girdling of lesions was transformed to logarithms and proportion of trees killed and proportion of successful *P. cinnamomi* isolations were transformed to arcsin square root values to homogenize the variance. For each host in experiment 1, the lesion lengths for the two roots per tree were averaged and analysed as a factorial experiment with rate and

concentration as fixed factors in the ANOVA. Lesion development and isolation of *P. cinnamomi* for each host in experiment 2, was analysed using a mixed model ANOVA with replicates within assessment time as a random effect and assessment time and concentration fixed effects. ANOVA of mortality data was computed with assessment time and concentration as fixed effects. In experiment 3, because the same trees were measured at each assessment time, lesion development was analysed at each assessment time, with concentration as a fixed effect in the ANOVA. Paired *t*-test was used to compare differences between injected and not injected trees along disease fronts. Where appropriate the Pearson correlation coefficient was calculated for a measure of association between variables. Significance was determined at $p \leq 0.05$.

3 Results

3.1 Sites

Nutrient levels in *E. marginata* forest soils were mainly higher than those from *B. coccinea* woodland soils (Table 1). Soil pH was slightly acidic, with forest soils being less acidic than woodland soils. In woodland, the nutrient levels of soil at 40–60 cm depth were less than surface soil, but the reverse tended to be the case for forest soils (Table 1). Phosphorus levels in the soils of all sites were low, being <35 μg/g.

3.2 Phosphite concentration on lesion development in roots of *B. grandis* and *E. marginata* (experiment 1)

Lesion length was significantly less in roots of *B. grandis* trees that had been stem injected with three concentrations of phosphite compared with the not injected control (Fig. 1). With the exception of *B. grandis* trees injected with 50 g phosphite/l, lesion lengths for the high rate were not significantly different to the low rate.

In roots of *E. marginata*, lesion development in response to phosphite was different to that in roots of *B. grandis* (Fig. 1). Lesion length in *E. marginata* did not differ significantly between phosphite concentration and rate. At the low rate, lesion length decreased with increasing phosphite concentration. At the high rate the relationship between phosphite concentration and lesion development was a U-shaped non-linear response, with lesion length decreasing as concentration increased from 0 to 50 g phosphite/l, but increased with phosphite concentrations >50 g phosphite/l (Fig. 1).

Phosphite injection resulted in brown staining of the wood around the injection site, especially at 200 g phosphite/l. For concentrations <100 g phosphite/l there was no lasting effect of injection and drilled holes had callused over within a year after injection. *Banksia grandis* tolerated high concentrations of stem injected phosphite with no relationship between phosphite concentration and crown density rating (Fig. 2). In *E. marginata* leaf burn, leaf drop and production of stunted leaves resulted in a significant negative relationship between injected phosphite concentration and crown density rating (Fig. 2):

$$\text{Crown density} = 4.1 - 0.008 \text{ Phosphite concentration}, \quad r^2 = 0.98.$$

3.3 Phosphite concentration on lesion development in stems of *B. grandis* and *E. marginata* (experiment 2)

Lesions were significantly shorter in *B. grandis* stems that were injected with phosphite than in those that were not (Fig. 3a). Up to 1 year after injection, lesion length in stems of *B. grandis* was similar for all concentrations of phosphite injected. However, between 1 and

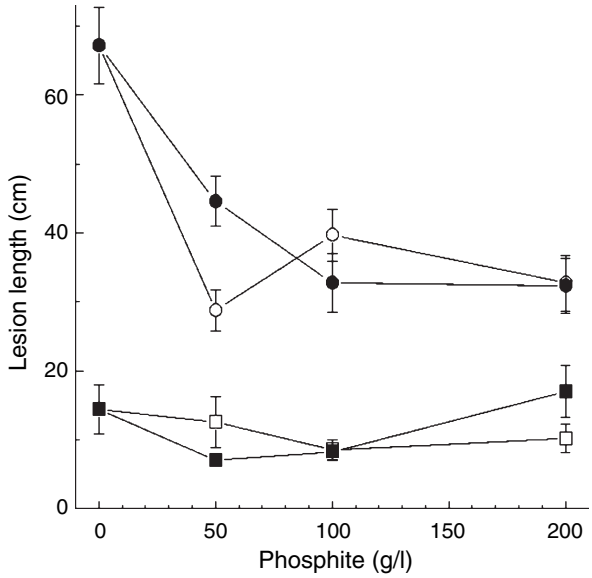

Fig. 1. Mean lesion length of *Phytophthora cinnamomi* (±SEM) 6 weeks after inoculation of roots of *Banksia grandis* and *Eucalyptus marginata* trees stem injected with two rates and four concentrations of phosphite. Roots of *B. grandis* (○) and of *E. marginata* (□) trees injected with 1 ml phosphite/cm stem circumference. Roots of *B. grandis* (●) and of *E. marginata* (■) trees injected with 2 ml phosphite/cm stem circumference. Lesions were allowed to develop for 3 weeks in the roots before stem injection with phosphite. Blank inoculations were 0.6 cm for *B. grandis* and 0.9 cm for *E. marginata*

4.3 years, differences between concentrations were apparent. Lesion length was shortest in stems injected with 100 g phosphite/l, intermediate in stems injected with 25 and 50 g phosphite/l and longest in stems that were not injected or injected with 12.5 g phosphite/l (Fig. 3a). At 4.3 years after injection there was a steep significant negative linear relationship between injected phosphite concentration and lesion length (Fig. 3a). The longest lesions developed in not injected stems and shortest lesions in stems injected with 100 g phosphite/l:

$$\text{Lesion length} = 30.8 - 0.22\ \text{Phosphite concentration}, r^2 = 0.99.$$

The effectiveness of phosphite concentration in controlling lesion length in *E. marginata* (Fig. 4a) was mainly similar to that described for *B. grandis*. Lesions were significantly shorter in *E. marginata* stems that were injected with phosphite than in those that were not. Up to 1 year after injection, lesion length in stems of *E. marginata* was similar for all concentrations of phosphite injected. However, between 1 and 4.3 years, differences between concentrations were apparent. Lesion length was shortest in stems injected with 25 and 100 g phosphite/l, intermediate in stems injected with 12.5 and 50 g phosphite/l and longest in stems that were not injected with phosphite (Fig. 4a). At 4.3 years after injection there was no significant linear relationship between injected phosphite concentration and lesion length because of the shorter lesions at 25 than 12.5 and 50 g phosphite/l (Fig. 4a).

Lesion girdling was significantly less in *B. grandis* stems injected with phosphite than in not injected stems (Fig. 3b). Six weeks after injection, all phosphite concentrations

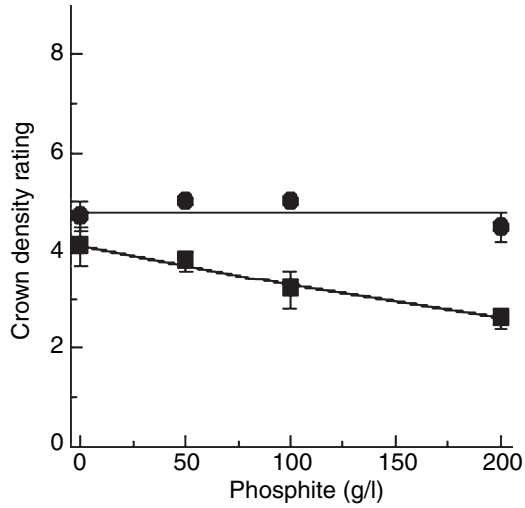

Fig. 2. Relationship between concentration of phosphite injected into stems of *Banksia grandis* (●) and *Eucalyptus marginata* (■) and mean crown density rating (±SEM) 4 weeks after injection. The crown rating varied from a rating of 9 for very dense crowns, to 5 for average and 1 for very sparse crowns

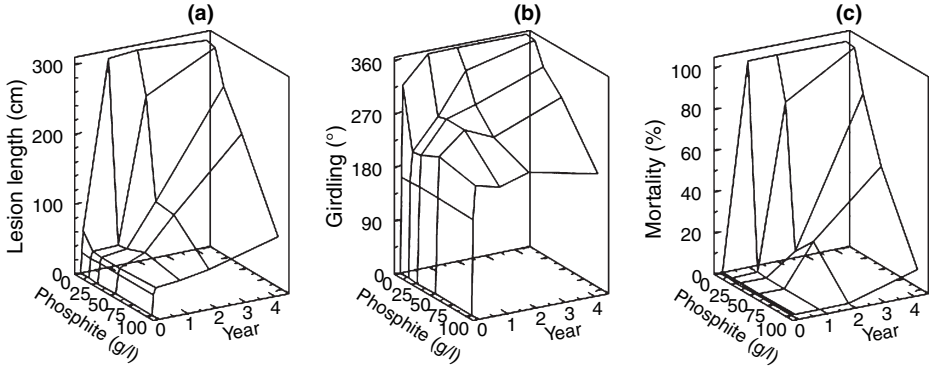

Fig. 3. Time course of (a) lesion length (b) girdling and (c) tree mortality following inoculation of stems of *Banksia grandis* with *Phytophthora cinnamomi* and either not injected or injected with four concentrations of phosphite and assessed up to 4.3 year after injection. Lesions were allowed to develop for 3 weeks before stem injection with phosphite. In blank inoculations, tissue necrosis was initially 2 cm by 22° and the cut had completely healed within a year

controlled girdling to a similar extent. However after 6 weeks, differences between concentrations increased with time. Lesion girdling was the least for *B. grandis* stems injected with 100 g phosphite/l, intermediate in stems injected with 25 and 50 g phosphite/l and greatest for stems not injected or injected with 12.5 g phosphite/l, (Fig. 3b). Figure 7 shows containment of lesions by callus tissue, 1 year after injection of stems with 100 g phosphite/l. By 4.3 years after injection there was a steep significant negative linear

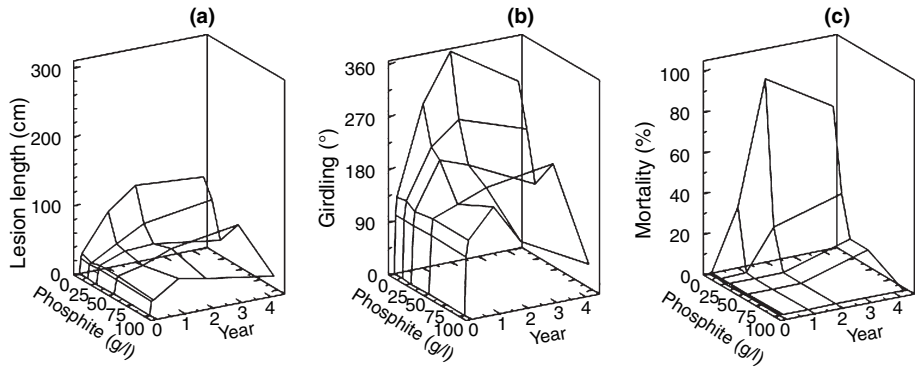

Fig. 4. Time course of (a) lesion length (b) girdling and (c) tree mortality following inoculation of stems of *Eucalyptus marginata* with *Phytophthora cinnamomi* and either not injected or injected with four concentrations of phosphite and assessed up to 4.3 year after injection. Lesions were allowed to develop for 3 weeks before stem injection with phosphite. In blank inoculations, mean tissue necrosis was initially 2.2 cm by 17° and the cut had completely healed within a year

relationship between injected phosphite concentration and girdling, with greatest girdling in not injected stems and least in stems injected with 100 g phosphite/l:

$$\text{Girdling} = 369.2 - 1.70\ \text{Phosphite concentration}, r^2 = 0.98.$$

The effectiveness of phosphite concentration in controlling lesion girdling in *E. marginata* was similar to that described for *B. grandis* (Fig. 4b). By 4.3 years after injection there was a steep significant negative linear relationship between injected phosphite concentration and girdling, with greatest girdling in not injected stems and least in stems injected with 100 g phosphite/l:

$$\text{Girdling} = 238.7 - 1.90\ \text{Phosphite concentration}, r^2 = 0.79.$$

In both *B. grandis* and *E. marginata*, recovery of *P. cinnamomi* from lesion margins 1 year after injection, was significantly less in trees treated with phosphite than in not injected trees. Percentage recovery of *P. cinnamomi* from *B. grandis* ranged from 100% from not injected trees to 2%, 6%, 14% and 11% from trees injected with 12.5, 25, 50 and 100 g phosphite/l, respectively. Percentage recovery of *P. cinnamomi* from *E. marginata* ranged from 88% from not injected trees to 39%, 31%, 15% and 11% from trees injected with 12.5, 25, 50 and 100 g phosphite/l, respectively. Increasing numbers of dead plants after 1 year prevented further sampling for *P. cinnamomi* recovery.

Reduction of lesion extension, girdling, and of recovery of *P. cinnamomi* with increased phosphite concentration, was reflected in the amount of plant death (Figs 3c and 4c). Death of *B. grandis* and *E. marginata* with time was least for trees treated with 100 g phosphite/l and greatest for not injected trees. By 4.3 years after injection of *B. grandis* (Fig. 3c), there was a steep significant negative linear relationship between injected phosphite concentration and percentage of plant death:

$$\%\ \text{Plants dead} = 104.0 - 0.96\ \text{Phosphite concentration}, r^2 = 0.98.$$

For *E. marginata* (Fig. 4c), injected phosphite concentration was significantly negatively linearly related to the logarithm of the percentage of plants dead 4.3 years after injection:

$$\log(\%\text{Plants dead} + 0.5) = 4.1 - 0.05\ \text{Phosphite concentration}, r^2 = 0.95.$$

3.4 Phosphite concentration on lesion development in stems of *B. coccinea* (experiments 3 and 4)

In comparison with *B. grandis*, the effectiveness of phosphite concentration in controlling lesion extension and girdling in *B. coccinea* showed a U-shaped non-linear relationship to phosphite concentration (Fig. 5). Lesion extension and girdling with time was greatest for not injected stems, least for *B. coccinea* stems injected with 25 g phosphite/l and intermediate in stems injected with 50 and 100 g phosphite/l (Fig. 5a,b). As was the case for *B. grandis* and *E. marginata*, change of lesion extension and girdling with phosphite concentration was reflected in the amount of plant death (Fig. 5c). Death of *B. coccinea* with time was least for trees treated with 25 g phosphite/l, greatest for not injected trees and intermediate for those injected with 50 and 100 g phosphite/l.

When *B. coccinea* trees were injected with 100 g phosphite/l along a disease front at site 4, significantly more injected trees died with time than trees not injected with phosphite (Fig. 6a). Injected trees had necrotic areas around the point of injection, and appeared to be under stress. In contrast, when lower levels of phosphite were injected into trees at site 3, significantly more trees died when not injected, compared with trees injected with 25 and 50 g phosphite/l (Fig. 6b).

3.5 Lesion containment

Lesions in trees treated with phosphite were confined by callus tissue 1 year after injection (Fig. 7). Table 2 gives the proportion of trees with lesions confined by callus tissue 4.3 year after injection for *B. grandis* and *E. marginata* and 3.7 years after injection for *B. coccinea*.

The greatest confinement of lesions by callus formation occurred for *E. marginata* (Table 2). In *B. grandis* and *E. marginata* the proportion of trees with lesions confined by callus increased with injected phosphite concentration. Lesions were confined with callus in all but one *B. grandis* and all *E. marginata* injected with 100 g phosphite/l. In trees not injected with phosphite, callus formation was not associated with lesions in *B. grandis* and was associated with a lesion in only one *E. marginata* tree (Table 2).

As for *B. grandis*, no callus was associated with lesions in stems of *B. coccinea* not injected with phosphite (Table 2). Callus formation showed a non-linear response to

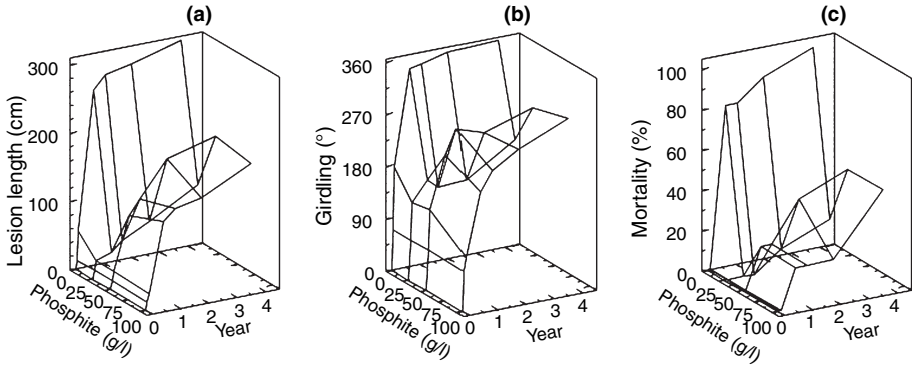

Fig. 5. Time course of (a) lesion length (b) girdling and (c) tree mortality following inoculation of stems of *Banksia coccinea* with *Phytophthora cinnamomi* and either not injected or injected with four concentrations of phosphite and assessed up to 3.7 year after injection. Lesions were allowed to develop for 3 weeks before stem injection with phosphite. In blank inoculations, mean tissue necrosis was initially 2.2 cm by 37° and the cut had completely healed within a year

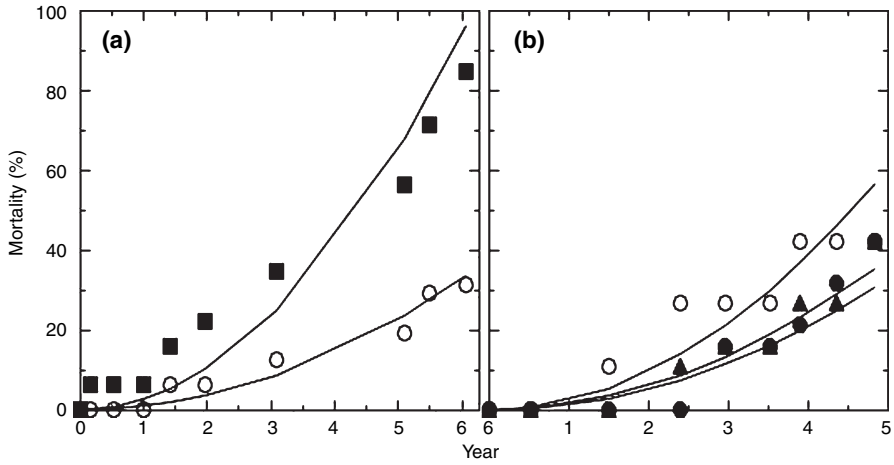

Fig. 6. Mortality of *Banksia coccinea* on *Phytophthora cinnamomi* disease fronts and either not injected or injected with phosphite. (a) Site 4 (Table 1) trees not injected (○, line: √mortality = 0.96 year, $r^2 = 0.97$) or injected with 100 g phosphite/l (■, line: √mortality = 1.62 year, $r^2 = 0.95$). (b) Site 3 (Table 1) trees not injected (○, line: √mortality = 1.55 year, $r^2 = 0.98$) or injected with 25 g phosphite/l (▲, line: √mortality = 1.23 year, $r^2 = 0.97$) or with 50 g phosphite/l (●, line: √mortality = 1.14 year, $r^2 = 0.90$)

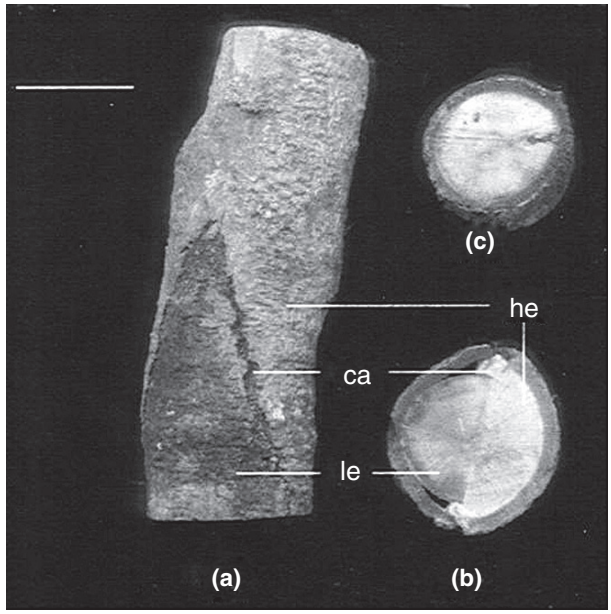

Fig. 7. Lesion development of *Phytophthora cinnamomi* 1 year after injection of stems of *Banksia grandis* and either injecting with 100 g phosphite/l (a, b) or not injected (c). Contained lesioned area following phosphite injection (a) showing callus formation (ca) at the interface of lesioned (le) and healthy (he) tissue. Transverse section (b) showing containment of lesion (le) by callus (ca) tissue. Inoculated stem of tree not injected with phosphite was completely girdled by *P. cinnamomi* (c). Bar = 1 cm

Table 2. **Proportion of 10 trees showing confinement of lesion by callus tissue (Fig. 7) following inoculation of stems of *Banksia grandis*, *Eucalyptus marginata* and *B. coccinea* with *Phytophthora cinnamomi* and either not injected or injected with different concentrations of phosphite. Assessment was 4.3 years after injection for *B. grandis* and *E. marginata* and 3.7 years after injection for *B. coccinea*. Blank inoculations had completely healed within a year**

	Phosphite concentration (g/l)				
Host	0	12.5	25	50	100
Banksia grandis	0	0	0.2	0.5	0.9
Eucalyptus marginata	0.1	0.4	0.7	0.6	1.0
B. coccinea	0	-[1]	0.8	0.5	0.5

[1]No 12.5 g of phosphite/l treatment for *B. coccinea*.

phosphite with most lesions callused in trees injected with 25 g phosphite/l, intermediate numbers of lesions callused in trees injected with 50 and 100 g phosphite/l and no callus associated with lesions in trees not injected.

4 Discussion

Phosphite was associated with effective control of lesion development by *P. cinnamomi* in roots of *B. grandis* following trunk injection. Although phosphite did not control lesion development by *P. cinnamomi* in roots of *E. marginata*, the test of phosphite effectiveness was severe as *P. cinnamomi* was allowed to invade the root tissue for 3 weeks before stem injection. Phosphite effectiveness needs further testing in *E. marginata* roots by injecting the trees before challenge inoculation with the pathogen. In experiments 2 and 3, phosphite effectiveness was determined by inoculating stems rather than roots because of the impracticality of inoculating the large number of roots required. Root size and number vary greatly between plants and labour requirements for preparing roots for sufficient replication are prohibitive (Shearer et al. 1988). Roots of *B. grandis* and *E. marginata* have shown the same relative susceptibilities to *P. cinnamomi* invasion as stems (Shearer et al. 1987a; Tippett et al. 1989). However the effectiveness of phosphite has been little investigated in roots and more testing is needed across a range of plant species.

Phytotoxic symptoms occurred in crowns and stems following phosphite injection. Leaf burn, leaf drop and production of stunted leaves, similar to that reported in horticulture crops (Wicks and Hall 1988; Holderness 1990), resulted in a negative linear relationship between injected phosphite concentration and crown density rating of *E. marginata*. *Banksia grandis* was much more tolerant of high concentrations of phosphite than *E. marginata*, with no relationship between injected phosphite concentration and crown density rating. Phosphite injection resulted in brown discolouration at the injection point similar to that observed in horticulture (Darvas et al. 1984; Long et al. 1989; Guest et al. 1994). As in the horticulture situation, no lasting damage occurred when dosage was <100 g phosphite/l.

Phosphite has a complex mode of action in plant tissues (Guest and Grant 1991). Smillie et al. (1989) found a mixed mode of action of phosphite in plant tissues. The site of action of phosphite was considered to be in the pathogen with the defence mechanisms of the host plant playing a critical role in arresting pathogen development.

Containment of *P. cinnamomi* by callus tissue following phosphite injection (Fig. 7) suggests stimulated coordinated responses of treated tissues at the time of injection. Alternately girdling of stems by *P. cinnamomi* in tissue not treated with phosphite indicated that dynamic defences were either not initiated or were slower than pathogen

activity in the absence of phosphite. No callus tended to occur in not treated plants, similar to observations of SMITH et al. (1997). Active resistant responses such as formation of callus tissue that were expressed in *B. grandis* in response to *P. citricola* invasion (SHEARER et al. 1988), were stimulated against *P. cinnamomi* following phosphite injection. *Eucalyptus marginata* has known active defence responses to *P. cinnamomi* invasion (TIPPETT et al. 1983, 1985), which were stimulated by phosphite injection. Our finding that stimulated host defence responses were associated with effective control of *P. cinnamomi* for at least 4 years following phosphite injection, supports the conclusion of SMILLIE et al. (1989) and EL-HAMALAWI et al. (1995) that well-developed dynamic defence systems in the plant favour effective control by phosphite.

Initial control of lesion development, at 12.5 g phosphite/l in *B. grandis* and *E. marginata*, then expansion of lesions after 1 year is analogous to the renewed advance, or break-out, of *P. cinnamomi* from confined lesions observed by TIPPETT et al. (1983, 1985). Presumably weaker or incomplete barriers of lesion confinement were formed at 12.5 g phosphite/l than at higher concentrations of injected phosphite. Alternately low concentrations of phosphite may inhibit pathogen development by stimulating host defence enzymes (AFEK and SZTEJNBERG 1989; JACKSON et al. 2000). In this study such an effect was short lived with longevity related to phosphite concentration and host susceptibility. At high concentrations phosphite may act through direct inhibition of the pathogen (AFEK and SZTEJNBERG 1989) allowing establishment of host defence mechanisms (JACKSON et al. 2000). This is supported by the occurrence of a higher proportion of lesions confined by callus tissue in *B. grandis* and *E. marginata* stems injected with increasing phosphite concentration.

Effective control of *P. cinnamomi* infection occurred in moderately susceptible *E. marginata* stems injected with lower concentrations of phosphite than in susceptible *B. grandis*. Injected phosphite concentrations in the range of 12.5–100 g phosphite/l were associated with inhibited lesion development in inoculated *B. grandis* and *E. marginata* during the first year. While lesions started to expand after the first year in *E. marginata* injected with 12.5 g phosphite/l, lesions were effectively controlled during a 4.3-year period in stems injected with 25–100 g phosphite/l. However lesions in *B. grandis* injected with 12.5–50 g phosphite/l started to expand after the first year and lesions were only inhibited for the 4.3 years by 100 g phosphite/l. This indicates that in susceptible hosts such as *B. grandis*, the effectiveness of phosphite against *P. cinnamomi* must be monitored for more than 2 years in order to obtain a true estimate of long-term control. AFEK and SZTEJNBERG (1989) also found that control of *P. citrophthora* development in resistant citrus species occurred at lower concentrations of phosphite than in susceptible species.

Banksia coccinea showed a U-shaped non-linear response to phosphite in contrast to the linear relationships between lesion inhibition and injected phosphite concentration for *B. grandis* and *E. marginata*. Non-linear relationships between lesion inhibition and injected phosphite concentration for *B. coccinea* occurred both when phosphite effectiveness was determined by either artificial inoculation or natural infection of injected plants. Lesion length, girdling and mortality decreased as phosphite concentration increased from 0 to 25–50 g phosphite/l, but increased thereafter. SMITH (1994) also found a U-shaped non-linear relationship between lesion inhibition and phosphite concentration for *B. coccinea* following low-volume foliar spray of phosphite in a glasshouse environment. *Banksia coccinea* trees injected with 100 g phosphite/l along a disease front were under stress and this may have contributed to significantly more injected trees dying with time than trees not injected with phosphite. Similarly lesions increased in roots of *E. marginata* trees that showed considerable stress after injection with 200 g phosphite/l. Stress associated with concentrations of injected phosphite higher than a threshold possibly disrupts the mechanisms of phosphite effectiveness leading to decreased inhibition of pathogen development. Injection of a range of *Banksia* species (B. L. SHEARER, unpubl.

data) has shown that rough barked species such as *B. attenuata* and *B. grandis* can generally tolerate higher concentrations of injected phosphite than smoothed barked species such as *B. brownii*, *B. coccinea* and *B. prionotes*. Because of the non-linear response to phosphite of some plant species, whereas injected concentrations of up to 100 g phosphite/l would be recommended for *B. attenuata* and *B. grandis*, injected concentration for *B. brownii*, *B. coccinea* and *B. prionotes* should not exceed 50 g phosphite/l.

One injection of 50–100 g phosphite/l protected treated *Banksia* and Eucalypt trees for at least 4 years is similar to the finding of Shearer et al. (2004b) that injection of *B. attenuata* trees with 50 g phosphite/l reduced extension of a *P. cinnamomi* disease front for 5 years. These periods of protection following one injection of phosphite in native communities are much longer than those found in horticulture. Pegg et al. (1987) controlled *P. cinnamomi* infection of avocado with annual injections of 100 and 200 g phosphite/l. Injections of 186 g phosphite/l were needed to be repeated at 6-monthly intervals for control of *Phytophthora* diseases of cocoa (Guest et al. 1994). Schutte et al. (1991) recommended that citrus should be treated with repeated applications of phosphite within 0.1 year, regardless of the method of application. Differences in the longevity of action of phosphite between native plant and horticulture hosts will depend on differences in the dynamics of competing source, sink relationships at the time of injection (Wiley et al. 1995), the presence of active defence responses to *P. cinnamomi* invasion and environment interactions. Little is known of how these factors affect phosphite effectiveness in native communities or the horticulture situation.

Longevity of action of phosphite for 4–5 years in native plant species after one injection makes phosphite injection a practical control option for the control of *P. cinnamomi* disease front extension and the protection of threatened flora. Research into the effect of factors affecting longevity of action of phosphite would facilitate optimization of timing of injection.

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Résumé

Concentration efficace de phosphite pour lutter contre Phytophthora cinnamomi par injection de tronc chez des espèces de Banksia et Eucalyptus marginata

L'effet de la concentration de phosphite sur le développement des lésions de *Phytophthora cinnamomi* Rands dans les troncs et racines de *Banksia grandis* Wild. et *Eucalyptus marginata* Donn. ex Smith et les troncs de *B. coccinea* R. Br. a été étudié sur une période de 4.3 années après injection du phosphite dans les troncs. La longueur de lésion est significativement plus faible dans les racines de *B. grandis* ayant subi une injection 3 semaines après inoculation avec 3 concentrations de phosphite (50, 100 et 200 g de phosphite/l) à deux taux (1 et 2 ml/cm de circonférence du tronc) que chez les témoins non traités. A l'exception des *B. grandis* injectés avec 50 g de phosphite/l, la longueur des lésions pour le taux d'injection le plus fort est non significativement différente de celle obtenue pour le taux le plus faible. La réponse au phosphite est différente dans les racines de *E. marginata* par rapport à celles de *B. grandis*; la longueur de lésion n'est dans ce cas pas affectée significativement par la concentration de phosphite et le taux d'injection. La longueur de lésion et la ceinturation des troncs sont plus faibles chez les *B. grandis* et *E. marginata* injectés que chez les témoins. Un an après injection, les lésions sont efficacement contenues par les tissus de cicatrisation dans les troncs traités. Après 4.3 années, on observe une relation linéaire significative fortement négative entre la concentration de phosphite et la longueur de lésion ou la ceinturation, avec le plus fort développement dans les troncs non traités et le plus faible dans les troncs traités à 100 g de phosphite/l. Le réisolement de *P. cinnamomi* à la marge des lésions un an après injection est significativement plus faible dans les arbres traités avec le phosphite que dans les arbres non traités. Le niveau de mortalité reflète la limitation de l'extension des lésions et de la ceinturation, ainsi que le moindre réisolement de *P. cinnamomi* avec l'augmentation de la concentration en phosphite: on observe une relation linéaire significative fortement négative entre la

concentration de phosphite et le pourcentage de mortalité, 4.3 années après l'injection. Contrairement à *B. grandis* et *E. marginata*, une relation non linéaire est observée chez *B. coccinea* entre la concentration de phosphite et l'efficacité de limitation des lésions et de la ceinturation. Celle-ci est maximale pour les tiges de *B. coccinea* injectées à 25 g de phosphite/l, minimale pour les tiges non traitées, et intermédiaire pour les tiges traitées à 50 g et 100 g de phosphite/l. Comme chez *B. grandis* et *E. marginata*, la limitation des lésions et de la ceinturation en fonction de la concentration de phosphite se traduit en terme de niveau de mortalité. La réponse non- linéaire au phosphite suggère que, dans le cas de *B. coccinea*, la concentration de 50 g/l de phosphite ne devrait pas être dépassée pour les injections, alors que des concentrations allant jusqu'à 100 g/l pourrait être recommandées dans le cas de *B. grandis*. La persistance de l'action du phosphite pendant 4–5 ans dans des plantes indigènes après une seule injection montre que cette méthode peut être utilisée en pratique pour lutter contre l'extension du front de la maladie causée par *P. cinnamomi* et protéger la flore menacée. L'étude des facteurs affectant cette persistance pourrait permettre d'optimiser la période d'injection.

Zusammenfassung

Wirksame Konzentration von Phosphit zur Bekämpfung von Phytophthora cinnamomi durch Stamminjektion von Banksia-Arten und Eucalyptus marginata

Der Einfluss der Phosphit-Konzentration nach einer Injektion im Stamm auf die Bildung von Läsionen durch *Phytophthora cinnamomi* an Wurzeln und Stämmen von *Banksia grandis* und *Eucalyptus marginata* sowie an Stämmen von *Banksia coccinea* wurde während 4,3 Jahren untersucht. Die Länge der Läsionen war in Wurzeln von *B. grandis* mit einer Phosphitbehandlung (3 Wochen nach Inokulation der Wurzeln Stamminjektion mit 50, 100 und 200 g/l Phosphit in zwei Dosen von 1 und 2 ml/cm Stammumfang) signifikant kleiner als in unbehandelten Kontrollbäumen. Mit Ausnahme von 50 g/l Phosphit wirkte sich die höhere Dosis im Vergleich zur tieferen signifikant auf die Länge der Läsionen aus. An Wurzeln von *E. marginata* hatten dagegen weder die Phosphitkonzentration noch die beiden Dosen einen signifikanten Effekt auf die Läsionenbildung. Nach einer Stamminokulation bewirkten alle Phosphitbehandlungen kürzere Läsionen und weniger stammumfassende Nekrosen bei *B. grandis* und *E. marginata* im Vergleich zu den unbehandelten Kontrollen. Ein Jahr nach der Injektion waren die Läsionen an Stämmen mit Phosphitbehandlung durch Kallusgewebe abgegrenzt. Nach 4,3 Jahren ergab sich für beide Arten eine enge, signifikant negative lineare Beziehung zwischen der Phosphitkonzentration und der Länge und Breite der Nekrosen, mit den grössten Läsionen an unbehandelten Stämmen und den kleinsten an solchen, die mit 100 g/l Phosphit behandelt worden waren. *P. cinnamomi* wurde aus den Läsionsrändern ein Jahr nach der Injektion bei behandelten Bäumen im Vergleich zur Kontrolle signifikant weniger häufig nachgewiesen. Die Begrenzung der Läsionen und die Reduktion von *P. cinnamomi* wirkte sich auch auf die Mortalität aus: 4,3 Jahre nach der Injektion ergab sich eine enge, signifikant negative lineare Beziehung zwischen der Phosphitkonzentration und dem Prozentsatz abgestorbener Bäume. Im Gegensatz zu *B. grandis* und *E. marginata* ergab sich für *B. coccinea* eine U-förmige nicht lineare Beziehung zwischen den Phosphitkonzentrationen und der Wirksamkeit der Behandlung. Hinsichtlich des Wachstums der Läsionen und des Ringelungseffektes war die Behandlung mit 25 g/l Phosphite am wirksamsten, 50 und 100 g/l waren intermediär und Bäume ohne Injektion reagierten am wenigsten. Dies galt auch für die Mortalität. Aufgrund der nicht linearen Reaktion einiger Pflanzenarten auf Phosphit sollten auch bei *B. coccinea* 50 g/l Phosphit nicht überschritten werden, bei *B. grandis* werden 100 g/l empfohlen. Die lange Wirksamkeit von 4–5 Jahren bei einheimischen Pflanzenarten nach nur einer Injektion lässt Phosphite als geeignet erscheinen, um die Ausbreitung von *P. cinnamomi* an einer Krankheitsfront zu bekämpfen und die gefährdete Flora zu schützen. Untersuchungen zu Einflussfaktoren auf die Dauer der Phosphitwirkung könnten die Optimierung des Injektionszeitpunktes erleichtern.

References

Aberton, M. J.; Wilson, B. A.; Cahil, M. J., 1999: The use of potassium phosphonate to control *Phytophthora cinnamomi* in native vegetation at Anglesea, Victoria. Australas. Plant Pathol. **28,** 225–234.

Afek, U.; Sztejnberg, A., 1989: Effects of fosetyl-Al and phosphorous acid on scoparone, a phytoalexin associated with resistance of citrus to *Phytophthora citrophthora*. Phytopathology **79,** 736–739.

Averbuch-Pochot, M. T.; Durif, A., 1996: Topics in Phosphate Chemistry. Singapore: World Scientific.

Cohen, Y.; Coffey, M. D., 1986: Systemic fungicides and the control of Oomycetes. Annu. Rev. Phytopathol. **24,** 311–338.

Darvas, J. M.; Toerien, J. C.; Milne, D. L., 1984: Control of avocado root rot by trunk injection with phosethyl-Al. Plant Dis. **68,** 691–693.

Day, P. R., 1965: Particle fraction and particle-size analysis. In: Methods of Soil Analysis. Part 1. Agronomy Monograph 9. Ed. by Black, C. A. Madison Wisconsin: ASA and SSSA, pp. 545–567.

El-Hamalawi, Z. A.; Menge, J. A.; Adams, C. J., 1995: Methods of fosetyl-Al application and phosphonate levels in avocado tissue needed to control stem canker caused by *Phytophthora citricola*. Plant Dis. **79,** 770–778.

Grimes, R. F., 1987: Crown assessment of natural spotted gum – ironbark forest. Technical Paper 7. Brisbane: Queensland Department of Forestry.

Guest, D.; Grant, B., 1991: The complex action of phosphonates as antifungal agents. Biol. Rev. **66,** 159–187.

Guest, D.; Anderson, R. D.; Foard, H. J.; Phillips, D.; Worboys, S.; Middleton, R. M., 1994: Long-term control of *Phytophthora* disease of cocoa using trunk- injected phosphonate. Plant Pathol. **43,** 479–492.

Hardy, G. E. StJ.; Barrett, S.; Shearer, B. L., 2001: The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. Australas. Plant Pathol. **30,** 133–139.

Holderness, M., 1990: Efficacy of neutralised phosphonic acid (phosphorous acid) against *Phytophthora palmivora* pod rot and canker of cocoa. Australas. Plant Pathol. **19,** 130–131.

Jackson, T. J.; Burgess; T.; Colquhoun; I. StJ.; Hardy; G. E., 2000: Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. Plant Pathol. **49,** 147–154.

Kirby, K. N., 1993: Advanced Data Analysis with SYSTAT. New York: Van Nostrad Reinhold.

Lim, T. M., 1993: Trunk injection with phosphorous acid for controlling *Phytophthora* on chestnuts: early promising results. In: Abstract, 9th Biennial Conference. Australasian Plant Pathology Society, Hobart July 4-8, Tasmania. Hohart: Australia, pp. 25.

Long, P. G.; Miller, S. A.; Davis, L. K., 1989: Duration of fungicidal effect following injection of apple trees with fosetyl-Al. J. Phytopathol. **124,** 89–96.

McKenzie, H. A.; Wallace, H. S., 1954: The Kjeldahl determination of nitrogen: a critical study of digestion conditions – temperature, catalyst and oxidising agent. Aust. J. Chem. **7,** 55–70.

Murphy, J.; Riley, T., 1962: A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta **27,** 31–36.

Pegg, K. G.; Whiley, A. W.; Langdon, P. W.; Saranah, J. B., 1987: Comparison of fosetyl-Al, phosphorous acid and metalaxyl for the long-term control of *Phytophthora* root rot of avocado. Aust. J. Exp. Agric. **27,** 471–474.

Piper, C. S., 1942: Soil and Plant Analysis. Adelaide: University of Adelaide.

Schutte, G. C.; Bezuidenhout, J. J.; Kotze, J. M., 1991: Timing of application of phosphonate fungicides using different application methods as determined by means of gas-liquid-chromatography for *Phytophthora* root rot control of citrus. Phytophylactica **23,** 69–71.

Shea, S. R.; Gillen, K. J.; Leppard, W. I., 1980: Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in soil in diseased, freely drained *Eucalyptus marginata* Sm sites in the northern jarrah forest of south-western Australia. Prot. Ecol. **2,** 135–156.

Shearer, B. L., 1990: Dieback of native plant communities caused by *Phytophthora* species – a major factor affecting land use in south-western Australia. Land Water Res. News **5,** 15–26.

Shearer, B. L., 1994: The major plant pathogens occurring in native ecosystems of south-western Australia. J. R. Soc. West. Aust. **77,** 113–122.

Shearer, B. L.; Fairman, R. G., 1991: Control of *Phytophthora cinnamomi* species in native communities with phosphorous acid. In: Proceedings of the Conservation Biology in Australia and Oceania Conference. Brisbane: University of Queensland, pp. 72.

Shearer, B. L.; Fairman, R. G., 1997: Foliar application of phosphite delays and reduces the rate of mortality of three *Banksia* species in communities infested with *Phytophthora cinnamomi*. In: Proceedings of the 11th Biennial Conference of the Australasian Plant Pathology Society. Perth: Australasian Plant Pathology Society, pp. 180.

Shearer, B. L.; Tippett, J. T., 1989: Jarrah dieback: the dynamics and management of *Phytophthora cinnamomi* in the jarrah (*Eucalyptus marginata*) forest of south-western Australia. Research Bulletin 3. Perth: Department of Conservation and Land Management.

Shearer, B. L.; Michaelsen, B. J.; Warren, H. J., 1987a: Comparative behaviour of *Phytophthora* species in the secondary phloem of stems and excised roots of *Banksia grandis* and *Eucalyptus marginata*. Aust. J. Bot. **35,** 103–110.

Shearer, B. L.; Shea, S. R.; Deegan, P. M., 1987b: Temperature-growth relationships of *Phytophthora cinnamomi* in the secondary phloem of roots of *Banksia grandis* and *Eucalyptus marginata*. Phytopathology **77,** 661–665.

Shearer, B. L.; Michaelsen, B. J.; Somerford, P. J., 1988: Effects of isolate and time of inoculation on invasion of secondary phloem of *Eucalyptus* spp. and *Banksia grandis* by *Phytophthora* spp. Plant Dis. **72,** 121–126.

Shearer, B. L.; Crane, C. E.; Cochrane, A., 2004a: Quantification of the susceptibility of the native flora of the south-west botanical province, Western Australia, to *Phytophthora cinnamomi*. Aust. J. Bot. **52,** 435–443.

Shearer, B. L.; Crane, C. E.; Fairman, R. G., 2004b: Phosphite reduces disease extension of a *Phytophthora cinnamomi* front in *Banksia* woodland, even after fire. Australas. Plant Pathol. **33,** 249–254.

Smillie, R.; Grant, B. R.; 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.

Smith, B., 1994: Effects of phosphonic acid and sodium silicate on lesion development of *Phytophthora cinnamomi* and histological responses in host species endemic to Western Australia. Honours Thesis. Perth: University of Western Australia.

Smith, B. J.; Shearer, B. L.; Sivasithamparam, K., 1997: Compartmentalization of *Phytophthora cinnamomi* in stems of highly susceptible *Banksia brownii* treated with phosphonate. Mycol. Res. **101,** 1101–1107.

Tippett, J. T.; Shea, S. R.; Hill, T. C.; Shearer, B. L., 1983: Development of lesions caused by *Phytophthora cinnamomi* in the secondary phloem of *Eucalyptus marginata*. Aust. J. Bot. **31,** 197–210.

Tippett, J. T.; Hill, T. C.; Shearer, B. L., 1985: Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi*. Aust. J. Bot. **33,** 409–418.

Tippett, J. T.; Crombie, D. S.; Hill, T. C., 1987: The effect of phloem water relations on the growth of *Phytophthora cinnamomi* in *Eucalyptus marginata*. Phytopathology **77,** 246–250.

Tippett, J. T.; McGrath, J. F.; Hill, T. C., 1989: Site and seasonal effects on susceptibility of *Eucalyptus marginata* to *Phytophthora cinnamomi*. Aust. J. Bot. **37,** 481–490.

Tsao, P. H.; Guy, S. O., 1977: Inhibition of *Mortierella* and *Pythium* in *Phytophthora*-isolation medium containing hymexazol. Phytopathology **67,** 796–801.

Wicks, T. J.; Hall, B., 1988: Preliminary evaluation of phosphorous acid, fosetyl- Al and metalaxyl for controlling *Phytophthora cambivora* on almond and cherry. Crop Prot. **7,** 314–318.

Wiley, A. W.; Hargreaves, P. A.; Pegg, K. G.; Doogan, V. J.; Ruddle, L. J.; Saranah, J. B.; Langdon, P. W., 1995: Changing sink strengths influence translocation of phosphonate in avocado (*Persea americana* Mill.) trees. Aust. J. Agric. Res. **46,** 1079–1090.

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