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Application of phosphite in a high-volume foliar spray delays and reduces the rate of mortality of four *Banksia* species infected with *Phytophthora cinnamomi*

B. L. Shearer[A,B] *and R. G. Fairman*[A]

[A]Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

[B]Corresponding author. Email: Bryan.Shearer@dec.wa.gov.au

Abstract. There were two experiments in which three phosphite concentrations were sprayed onto the foliage of either *Banksia brownii*, *B. baxteri* or *B. coccinea* naturally infected with *Phytophthora cinnamomi* in the Esperance Plains bioregion of Western Australia, or *B. grandis* artificially infected with *P. cinnamomi* in *Eucalyptus marginata* forest of south-western Australia. Plots were either sprayed with one of three concentrations of phosphite (2.5, 5 or 10 g phosphite/L) and penetrant-spreading agent or sprayed only with penetrant-spreading agent. Mortality of the *Banksia* species, understorey and crown health, plant height and soil inoculum at each site were the dependent variables. There were no significant differences in overstorey phytotoxic necrosis rating of *B. brownii*, *B. baxteri* and *B. coccinea* between phosphite treatments 2.7 and 4 years after the first spray. The greatest phytotoxic effect of phosphite application was the stunting of *B. coccinea* growth, which did not occur in *B. brownii*, *B. baxteri* and *B. grandis*. For naturally infested *Banksia* species, greatest mortality occurred in *B. brownii* and least in *B. coccinea*, with *B. baxteri* being intermediate between mortality for *B. brownii* and *B. coccinea*. There was a significant reduction in apparent mortality rate and increase in years to 50% mortality following foliar application of phosphite to the four *Banksia* species. Greatest reduction in mortality of the *Banksia* species occurred for concentrations between 5–10 g phosphite/L. Challenge inoculation showed that spray treatments of 5 g and 10 g of phosphite/L were effective in *B. grandis*, 2.2 years after spray. The proportion of positive *P. cinnamomi* soil samples from the top 3 cm of soil was significantly greater than the proportion positive from 30 cm below the soil surface in infested *B. brownii* and *B. coccinea*, but there was no significant difference in the proportion of positive *P. cinnamomi* soil samples between depth of sampling for *B. baxteri*. Phosphite had no significant effect on the frequency of isolation of *P. cinnamomi* from the soil of infested *B. baxteri* and *B. coccinea*. Determination of phosphite effectiveness against *P. cinnamomi* and phytotoxic responses between plant species will assist prescription optimisation for the most effective protection of threatened flora.

Additional keywords: disease progress analysis, phosphonate, predicted response surfaces, undulating mortality curve.

Introduction

The South-West Botanical Province of Western Australia is an internationally recognised biodiversity hotspot (Myers *et al*. 2000). *Phytophthora cinnamomi* is a major threat to the conservation of native plant communities within the province, as mortality following infestation by the pathogen results in reduced floristic diversity and a conspicuous decline in biomass (Shearer *et al*. 2007). Within the province current strategies aimed at conserving flora threatened by *P. cinnamomi* integrate delaying tactics such as hygiene (Shearer and Tippett 1989), long-term *ex situ* seed conservation (Cochrane and Coates 1994) and translocations (Monks and Coates 2002) with application of the systemic fungicide potassium phosphite (Shearer and Tippett 1989; Barrett 2005; Shearer *et al*. 2007).

Application of phosphite has proven effective in controlling *P. cinnamomi* infection in a range of native plant communities in south-western Australia (Shearer and Tippett 1989; Pilbeam *et al*. 2000; Tynan *et al*. 2001; Barrett 2003; Shearer *et al*. 2004, 2006; Shearer and Fairman 2007). Phosphite protected *Banksia attenuata*, *B. coccinea*, *B. grandis* and *Eucalyptus marginata* from *P. cinnamomi* for at least 4 years following trunk injection with between 50 and 100 g phosphite/L (Shearer *et al*. 2004, 2006; Shearer and Fairman 2007). Foliar application using a high-volume backpack sprayer at the rate of 5 g phosphite/L gave protection from *P. cinnamomi* for 0.5 to 2 years (Tynan *et al*. 2001). Low-volume aerial application of 24 kg phosphite/ha resulted in significantly greater survival of *P. cinnamomi*-susceptible species in sprayed areas for up to 3 years after spraying (Barrett 2003). However, effectiveness of phosphite for the control of *P. cinnamomi* infection of native plant species has mainly been tested following artificial infection of treated hosts (Smith 1994; Smith *et al*. 1997; Pilbeam *et al*. 2000; Tynan *et al*. 2001; Wilkinson *et al*. 2001*a*, 2001*b*; Barrett *et al*. 2003; Shearer *et al*. 2006; Shearer and Fairman 2007). The effects

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of phosphite on *P. cinnamomi* epidemic development in infested native communities has been little studied, other than monitoring of the effect of treatment on plant vigour (Aberton *et al*. 1999), mortality (Barrett 2003; Shearer *et al*. 2006; Shearer and Fairman 2007) and disease extension (Shearer *et al*. 2004).

As part of ongoing testing of phosphite application strategies (Shearer and Tippett 1989; Komorek *et al*. 1997; Barrett 2003; Shearer *et al*. 2004, 2006; Shearer and Fairman 2007), a trial was commenced to test the effect of a high-volume spray of phosphite on *Banksia* communities infested with *P. cinnamomi*. Epidemic development was assessed by measuring plant mortality and soil population levels of *P. cinnamomi* in infested *Banksia brownii*, *B. baxteri* and *B. coccinea* communities over time. Phosphite effectiveness was also tested following artificial infection of stems of *B. grandis* with *P. cinnamomi* and measuring plant mortality over time.

Materials and methods

Experimental design

There were two experiments in which three phosphite concentrations were sprayed onto the foliage of either naturally infected *Banksia brownii*, *B. baxteri* or *B. coccinea* in the Esperance Plains bioregion, or artificially infected *B. grandis* in *E. marginata* forest of south-western Australia.

Natural infection

The objective of the experiment was to evaluate the effect of high-volume foliar application of different phosphite concentrations on mortality of 10-year-old *B. brownii* and 15-year-old *B. baxteri* and *B. coccinea* in disease centres infested with *P. cinnamomi*. Treatments were replicated four times in a randomised block design along disease fronts at sites 1–3 (Table 1). Treatments were applied to 5 × 5 m plots along the disease front in each site, with one edge of the plot along the front and the rest of the plot in the apparently healthy area. Plots were sprayed with one of three concentrations of phosphite (2.5, 5 or 10 g phosphite/L) and penetrant-spreading agent or sprayed only with penetrant-spreading agent. Mortality of the *Banksia* species, phytotoxic necrosis of the overstorey, consisting of the uppermost canopy layer and the understorey under the canopy, plant height and soil inoculum at each site were the dependent variables.

Artificial infection

The objective of the experiment was to evaluate the effect of high-volume foliar application of phosphite concentrations on the mortality of 10-year-old *B. grandis* plants stem-inoculated with *P. cinnamomi*. Treatments were replicated four times in a randomised block design in 10 × 10 m plots along the contour in healthy forest above a *P. cinnamomi* disease front at site 4 (Table 1). Plot size was larger for *B. grandis* because of a lower density of plants than that for the other *Banksia* species. The plants were stem-inoculated with *P. cinnamomi* 3 weeks before phosphite application. Plots were sprayed with one of three concentrations of phosphite (2.5, 5 or 10 g phosphite/L) and penetrant-spreading agent, or only sprayed with penetrant-spreading agent. Mortality and crown health of inoculated

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

Characteristic	Site 1	Site 2	Site 3	Site 4
Banksia community	*B. brownii* shrubland	*B. baxteri* shrubland	*B. coccinea* shrubland	*E. marginata* forest
Latitude	34.8572°S	34.8839°S	34.9544°S	32.6394°S
Longitude	117.8103°E	118.4183°E	118.1103°E	116.0219°E
Local topography	Flat area	Mid-slope of old dune	Mid-slope of old dune	Upper slope
Aspect	South-west	North	South-west	South-west
Soil type	Laterite	Sand	Sand	Laterite
Soil at 0–10 cm				
Colour	Orange-brown	Grey-white	Grey-white	Brown
Coarse sand (%)	13.5	54.6	53.9	44.9
Fine sand (%)	78.6	41.8	38.9	45.5
Silt (%)	1.7	1.9	2.8	6.6
Clay (%)	6.2	1.7	4.4	3.0
Nitrogen (%)	0.08	0.09	0.11	0.05
Phosphorus (µg/g)	18.4	23.8	12.0	57.7
Potassium (µg/g)	15.2	33.7	23.9	59.7
Organic carbon (%)	2.4	1.4	3.8	2.0
pH	4.9	4.8	4.7	5.4
Soil at:	20 cm	50 cm	50 cm	50 cm
Colour	Brown-white	White	White	Brown
Coarse sand (%)	1.8	69.1	42.2	51.5
Fine sand (%)	82.9	29.3	56.1	37.1
Silt (%)	1.3	0.2	1.1	4.1
Clay (%)	14.0	1.4	0.6	7.3
Nitrogen (%)	0.04	0.02	0.02	0.07
Phosphorus (µg/g)	13.3	19.2	5.7	17.1
Potassium (µg/g)	21.0	18.4	<1.0	58.8
Organic carbon (%)	1.0	0.1	0.8	0.9
pH	5.2	4.9	4.6	5.7

B. grandis were the dependent variables. At the end of the experiment, 2.2 years after foliar spray with phosphite, 10 live plants per plot were further inoculated with *P. cinnamomi* in early January of 1993 (mid-summer), to determine the effectiveness of the spray treatment with the dependent variables being lesion length and girdling and recovery of the pathogen. Agreement between results from stem inoculation and natural infection suggest that stem inoculation of *B. grandis* would be a suitable bioassay for phosphite effectiveness against *P. cinnamomi* (Shearer and Fairman 2007).

Sites

Soil from each site was bulked, air-dried and analysed using the following methods. Phosphorus content was determined colourimetrically 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 1M potassium chloride solution and using the Kjeldahl method of McKenzie and Wallace (1954). The pH was determined from a 1 : 5 solution of soil and water. Percentages by weight of coarse (200–2000 µm) sand, fine (20–200 µm) sand, silt (2–20 µm) and clay (<2 µm) of the fine fraction were determined by the pipette method (Day 1965).

Stem inoculation

Trunks of *B. grandis* (diameter over bark of 1.2–7.3 cm, mean ± s.e. of 3.6 ± 0.1 cm) were wound-inoculated with isolate SC72 (IMI 264384) of *P. cinnamomi* in mid-November 1990 (late spring), using methods described previously (Shearer *et al*. 1987, 1988). Isolate SC72 was chosen because it was used in previous studies (Shea *et al*. 1980; Shearer *et al*. 1987, 1988, 2006; Shearer and Fairman 2007) 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 in a fresh cut in the phloem, 0.5 m above the soil surface. Blank inoculated controls were inoculated in a similar manner with sterile agar disks.

Spray

Six litres of phosphite solutions diluted from commercial formulation (Fos-Ject® 200, UIM Agrochemicals [Australia] Pty Ltd, active ingredient 200 g $H_2(PO_3H)$/L present as the mono-di potassium phosphite, adjusted to pH 5.7–6.0) and 0.2 g/L of an organo-silicone penetrant-spreading agent (Pulse®, Monsanto Australia Ltd, active ingredient 1000 g modified polydimethylsiloxane/L) were sprayed over the foliage in appropriate plots using a backpack sprayer with Hardi® flat spray nozzles. Preliminary trials had shown that 6 L per 5 × 5 m plot were sufficient to evenly cover the foliage without run-off to ground. The naturally infected plots were sprayed in early December 1989 for all three *Banksia* species and then again in late November 1991 for *B. brownii*, early August 1992 for *B. baxteri* and September 1992 for *B. coccinea*. For the inoculated *B. grandis*, the plots were sprayed in early December 1990, three weeks after initial inoculation.

Soil inoculum

On a line through the centre of each plot from the disease front edge to the back, permanent markers were placed 0.5 m from the front edge, in the middle of the plot and 0.5 m from the back edge. At each sampling time, soil samples were taken close to the permanent markers at 3 cm and 30 cm below the soil surface using a 4.5-cm-diameter King tube surface sterilised in 70% alcohol and dried between samples (Shearer and Shea 1987). At each sampling time two 1000-cm^3 samples were taken at each depth at each permanent marker to give six samples per depth for each plot. Sampling was done in early November 1989, 28 days before spraying, in early September 1990 and 1991, 0.8 and 1.8 years after spraying respectively, and in early August 1992, 2.7 years after the first spray. Soil samples were sealed in plastic bags and kept cool until processing within 2 days.

For each sample, a 100-cm^3 subsample was placed in a Styrofoam cup, covered with distilled water and baited with six cotyledons of *Eucalyptus sieberi* which were floated on the surface (Marks and Kassaby 1974). Baited cups were incubated at 25°C in a temperature-controlled glasshouse for 14 days. Cotyledons that indicated infection by undergoing a colour change from a normal reddish hue to green were plated onto selective medium (Tsao and Guy 1977). The presence of *P. cinnamomi* was determined by microscopic inspection of hyphal morphology and reproductive structures (Marks and Kassaby 1974). Samples negative for *P. cinnamomi* in the first baiting were rebaited with *E. sieberi* cotyledons. Soil inoculum was expressed as the percentage of samples positive for *P. cinnamomi* (Shearer and Shea 1987).

Assessment

Mortality

For mortality assessment, plants with all the leaves dead were considered killed by *P. cinnamomi*. In the case of inoculated *B. grandis*, some plants resprouted after the death of leaves, but observations outside the plots indicated that no resprouting occurred in naturally infected *B. brownii*, *B. baxteri* and *B. coccinea* once all the leaves of a plant died. Mortality of *B. brownii* was not continued after 4 years as most of the plants in non-sprayed plots were dead. Mortality assessment of *B. baxteri* and *B. coccinea* continued for 6 years. The tops of dead plants in naturally infested plots were removed following assessment, to assist counting of new dead plants in subsequent assessments and remove leaf necrosis due to infection from the phytotoxic necrosis rating. The inoculated *B. grandis* were labelled and new dead plants could be recognised without removing the tops of previous deaths.

Phytotoxicity

Phytotoxic necrosis of the *Banksia* overstorey layer and the understorey layer of the naturally infected sites was assessed 0.2, 2.7 and 4 years after spraying. The phytotoxic necrosis ratings were: 1 = 0–9% necrotic foliage, 2 = 10–19% necrotic foliage, 3 = 20–49% necrotic foliage, 4 = 50–74% necrotic foliage, 5 = 75–89% necrotic foliage, 6 = 90–99% necrotic foliage, and 7 = all plants dead. The height of 10 plants/plot was measured with a height stick 3.7 years after the first spray.

Lesions

Banksia grandis stems that were further inoculated 2.2 year after the phosphite spray were harvested 6 weeks after inoculation. 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 and tangential spread of the lesion at the point of inoculation was estimated. The presence of *P. cinnamomi* was verified by plating 10 pieces of tissue at the lesion margin onto selective medium (Tsao and Guy 1977).

Analysis

Apparent mortality rate was calculated from the regression of the logit of mortality against year after spray (Zadoks and Schein 1979). Years to 50% mortality was determined by dividing the constant by the slope coefficient (Shearer and Wilcoxson 1980).

For analysis of variance (ANOVA), assumptions of normality were checked by plotting residuals (Kirby 1993) and dependent variables were log-transformed to homogenise the variance. Phytotoxic necrosis rating and samples positive at each assessment time and apparent mortality rate and years to 50% mortality for naturally infested *Banksia* species were analysed as a factorial experiment with *Banksia* species and phosphite concentration as fixed factors and an interaction between the two in the ANOVA. For inoculated *B. grandis*, length and tangential spread of lesions were transformed to logarithms and proportion isolation of *P. cinnamomi* was transformed to arcsin square root values to homogenise the variance and analysed as one way ANOVA with phosphite concentration as a fixed factor. Where appropriate, the Pearson correlation coefficient was calculated for a measure of association between variables. Regression equations of the form:

$$\sqrt{\%\ \text{Mortality}} = \beta_0 + \beta_1\ \text{phosphite} + \beta_2\ \text{year} + \beta_3\ \text{phosphite}^2 + \beta_4\ \text{year}^2 + \beta_5\ \text{phosphite} \times \text{year}$$

were fitted to replicate means, where the dependent variable was % mortality of the naturally infected *Banksia* species and β_i the regression coefficients for the independent variables. Regression equations were determined using stepwise backward selection (Wilkinson 2002). Assumptions for linear regression were checked by plotting residuals (Kirby 1993). All coefficients were significant and retained in the model. Significance was determined at $P \leq 0.05$.

Results

Sites

Soil properties

The soil of the *B. brownii* community was a sandy gravel layer 30 cm or less over an impeding clay. *Banksia baxteri* and *B. coccinea* grew in deep white sand. The *B. grandis* grew in a Havel (1975) S vegetation site type gravel. Nutrient levels in the *E. marginata* forest soil tended to be higher than those from the *Banksia* shrubland soils (Table 1). Soil pH was slightly acidic, with forest soils being less acidic than woodland soils. The nutrient levels of soil at 40–60 cm depth were lower than at the surface soil. Phosphorus levels were low in all but the surface *E. marginata* soil, being less than 24 µg/g.

Plant density and initial mortality

For all *Banksia* species there was no significant difference in plant density between phosphite treatments. The number of plants per 25 m^2 plot was 13–46 (mean ± s.e. of 27 ± 3) for *B. browni*, 8–43 (mean ± s.e. of 17 ± 2) for *B. baxteri* and 14–113 (mean ± s.e. of 53 ± 7) for *B. coccinea*. There were 31–40 (mean ± s.e. of 36 ± 1) plants of *B. grandis* per 100 m^2 plot.

For all *Banksia* species there was no significant difference in initial plant mortality between phosphite treatments. Plant mortality at the time of spraying was 4–36% (mean ± s.e. of 22 ± 2%) for *B. brownii*, 0–43% (mean ± s.e. of 15 ± 3%) for *B. baxteri* and 0–27% (mean ± s.e. of 12 ± 2%) for *B. coccinea*.

Natural infection

Phytotoxicity

At 0.2 of a year after the first spray, the phytotoxic necrosis rating of the *Banksia* overstorey was significantly greater at 10 g phosphite/L than 2.5 and 0 phosphite/L, with the 5 g phosphite/L being intermediate between the two (Fig. 1). There were no significant differences in overstorey phytotoxic necrosis rating between phosphite treatments 2.7 and 4 years after the first spray. Phytotoxic necrosis rating of the understorey at 0.2 of a year and 2.7 years after the first spray was significantly greater at 10 g phosphite/L than 2.5 and 0 g phosphite/L, with the 5 g phosphite/L being intermediate between the two (Fig. 1). There were no significant differences in understorey phytotoxic necrosis rating between phosphite treatments 4 years after the first spray.

The greatest phytotoxic effect of phosphite application was the stunting of *B. coccinea* growth which did not occur in *B. brownii* and *B. baxteri* (Fig. 2). Plant height of *B. coccinea* significantly decreased as applied phosphite concentration increased from 2.5 to 10 g phosphite/L:

$$B.coccinea\ \text{plant height} = 296.13 - 6.56\ \text{applied phosphite concentration}\ (R^2 = 0.93).$$

Mortality

Mortality curves for the naturally infested *Banksia* communities were slightly undulating. The dead plants increased, then plateaued off, then increased again during the 4–6 years of monitoring (Fig. 3). Mortality curves show that *B. brownii* was more susceptible to *P. cinnamomi* than *B. baxteri* and *B. coccinea* (Fig. 3). All plants of *B. brownii* not treated with phosphite were killed by 4 years compared to over 6 years for extinction of *B. baxteri* and *B. coccinea* not treated with phosphite (Fig. 3). All three phosphite concentrations reduced mortality of the three *Banksia* species. Mortality started to increase by the second year after the first spray (Fig. 3) at which time the plants were resprayed.

Banksia brownii not sprayed with phosphite had the greatest apparent mortality rate, which was almost twice that for *B. baxteri* and *B. coccinea* (Table 2). The ANOVA *Banksia* species by phosphite treatment interaction term was not significant, indicating similar phosphite effect in the three

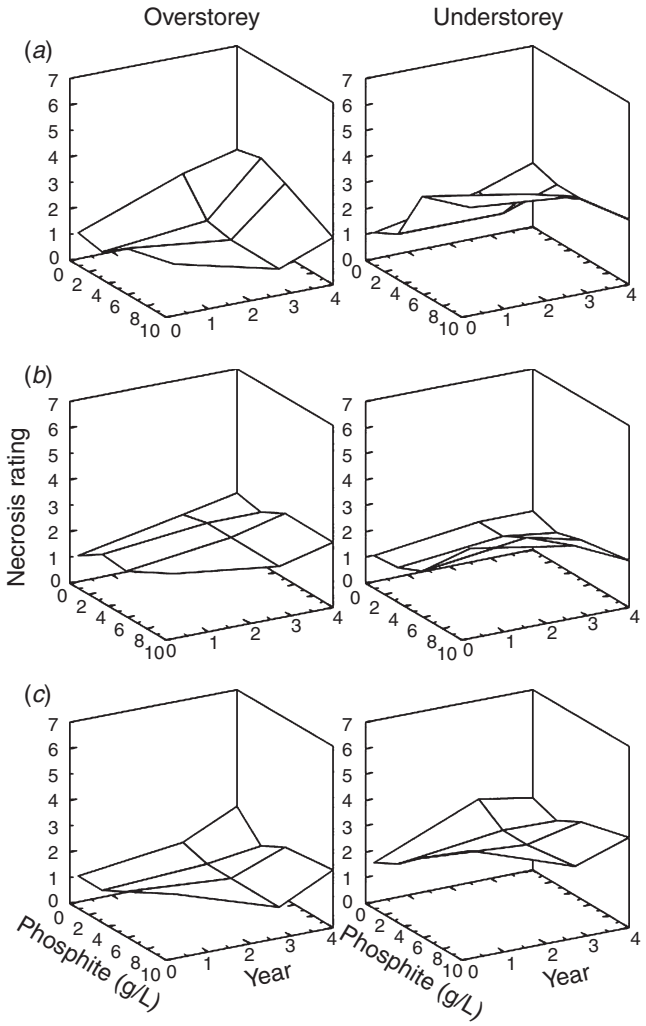

Fig. 1. Changes in phytotoxic necrosis rating of the *Banksia* overstorey and the understorey assessed three times after (*a*) *Banksia brownii*, (*b*) *B. baxteri* and (*c*) *B. coccinea* communities infested with *Phytophthora cinnamomi* were either not sprayed with phosphite or foliar sprayed with 2.5, 5 or 10 g phosphite/L.

Banksia communities. All three phosphite concentrations significantly reduced the apparent mortality rate (Table 2). Greatest reduction in apparent mortality rate occurred after application of 10 g phosphite/L with rates being reduced by a quarter to a third of plants not treated with phosphite (Table 2).

Trends in years to 50% mortality between *Banksia* species and phosphite treatment were similar to that described for apparent mortality rate (Table 2). The ANOVA interaction term was not significant, indicating similar phosphite effect in the three *Banksia* communities. *Banksia brownii* not sprayed with phosphite had the least years to 50% mortality, which was less than a half of that for *B. baxteri* and *B. coccinea* (Table 2). All three phosphite concentrations significantly increased the years to 50% mortality (Table 2). Greatest increase in years to 50% mortality occurred after application of 10 g phosphite/L, with times being ~5 fold greater than that for plants not treated

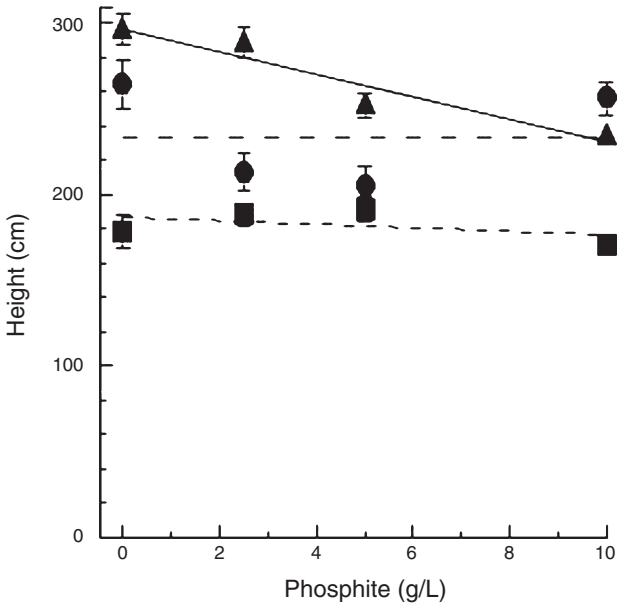

Fig. 2. The effect of phosphite concentration on mean live plant height (± s.e.) measured 3.7 years after *Banksia brownii* (●– –●), *B. baxteri* (■ - - ■) and *B. coccinea* (▲— ▲) communities infested with *Phytophthora cinnamomi* were either not sprayed with phosphite or foliar sprayed with 2.5, 5 or 10 g phosphite/L.

with phosphite (Table 2). There were significant correlation coefficients for the positive linear relationships between applied phosphite concentration and years to 50% mortality for *B. brownii* and *B. coccinea*:

$$\text{Years to 50\% } B.\,brownii \text{ mortality} = 0.94 + 0.52 \text{ applied phosphite concentration } (R^2 = 0.92);$$

$$\text{Years to 50\% } B.\,coccinea \text{ mortality} = 2.16 + 1.35 \text{ applied phosphite concentration } (R^2 = 0.95).$$

The predicted response surfaces clearly show the significant reduction in mortality following foliar application of phosphite (Fig. 4). Greatest predicted reduction in mortality with phosphite concentration and time occurred in *B. brownii*. Mortality was lowest in *B. coccinea* with that for *B. baxteri* being intermediate between mortality for *B. brownii* and *B. coccinea*. Greatest reduction in mortality of the *Banksia* species occurred for concentrations between 5–10 g phosphite/L (Fig. 4).

Soil inoculum

The effect of sampling position in the soil profile, sampling time and phosphite treatment on the frequency of isolation of *P. cinnamomi* varied between *Banksia* species. There was a significantly greater proportion of positive *P. cinnamomi* soil samples from infested *B. brownii* than *B. baxteri* with proportion of positive samples from *B. coccinea* being intermediate between the two (Fig. 5). The proportion of positive *P. cinnamomi* soil samples from the top 3 cm of soil was significantly greater than the proportion positive from 30 cm below the soil surface in infested *B. brownii* and *B. coccinea*. There was no significant

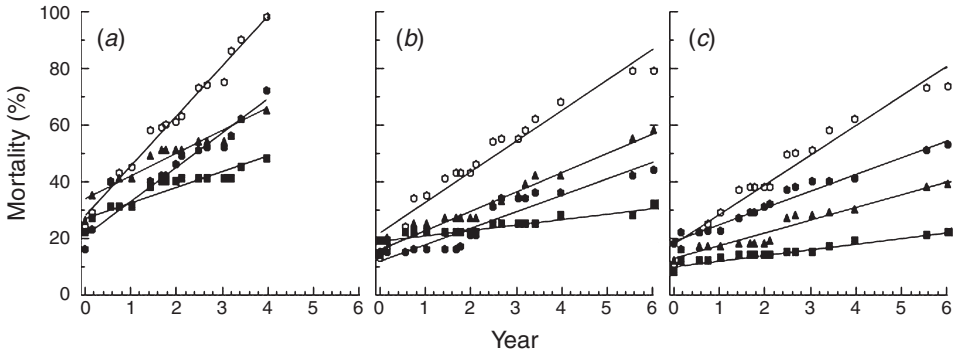

Fig. 3. Changes with year after spraying of percent mortality of (*a*) *Banksia brownii*, (*b*) *B. baxteri* and (*c*) *B. coccinea* plants in *Phytophthora cinnamomi* infested communities that were either not sprayed with phosphite (○) or foliar sprayed with 2.5 (●), 5 (▲) or 10 (■) g phosphite/L. Continuous lines indicate linear regressions with significant correlation coefficients for the mean values: (*a*) *B. brownii*: 0 g phosphite/L: % mortality = 27.89 + 17.62 year ($R^2 = 0.98$); 2.5 g phosphite/L: % mortality = 20.63 + 12.08 year ($R^2 = 0.97$); 5 g phosphite/L: % mortality = 33.87 + 7.91 year ($R^2 = 0.91$); 10 g phosphite/L: % mortality = 27.16 + 5.38 year ($R^2 = 0.86$). (*b*) *B. baxteri*: 0 g phosphite/L: % mortality = 21.62 + 10.97 year ($R^2 = 0.94$); 2.5 g phosphite/L: % mortality = 11.77 + 5.90 year ($R^2 = 0.87$); 5 g phosphite/L: % mortality = 17.08 + 6.59 year ($R^2 = 0.95$); 10 g phosphite/L: % mortality = 19.08 + 1.88 year ($R^2 = 0.90$). (*c*) *B. coccinea*: 0 g phosphite/L: % mortality = 17.88 + 10.52 year ($R^2 = 0.95$); 2.5 g phosphite/L: % mortality = 19.45 + 5.83 year ($R^2 = 0.97$); 5 g phosphite/L: % mortality = 12.56 + 4.56 year ($R^2 = 0.96$); 10 g phosphite/L: % mortality = 10.36 + 1.95 year ($R^2 = 0.94$).

Table 2. Mean (± s.e.) apparent mortality rate and years to 50% mortality of three *Banksia* species in *Phytophthora cinnamomi* disease centres either not sprayed with phosphite or foliar sprayed with 2.5, 5 or 10 g phosphite/L

Variable	*Banksia* species	Phosphite application (g/L) 0	2.5	5.0	10.0
Apparent mortality rate[A]	*B. brownii*	0.98 ± 0.15	0.67 ± 0.08	0.36 ± 0.08	0.24 ± 0.08
	B. baxteri	0.54 ± 0.08	0.27 ± 0.05	0.32 ± 0.06	0.14 ± 0.05
	B. coccinea	0.51 ± 0.04	0.31 ± 0.05	0.28 ± 0.03	0.16 ± 0.04
Years to 50% mortality	*B. brownii*	1.3 ± 0.6	2.4 ± 0.9	2.6 ± 0.8	6.6 ± 2.4
	B. baxteri	3.4 ± 0.7	5.9 ± 1.2	5.3 ± 0.9	16.0 ± 7.7
	B. coccinea	3.1 ± 0.5	5.5 ± 1.7	7.1 ± 1.2	16.6 ± 4.7

[A]/unit/year.

difference in proportion of positive *P. cinnamomi* soil samples between depth of sampling for *B. baxteri* (Fig. 5). Isolation of *P. cinnamomi* was most frequent from soil of infested *B. brownii* 1.8 years after the first spray and least frequent 0.8 and 2.7 years after the first spray. For soil from infested *B. baxteri* and *B. coccinea*, *P. cinnamomi* was most frequently isolated 28 days before and 0.8 of a year after the first spray and least frequently isolated 1.8 and 2.7 years after the first spray. Phosphite had no significant effect on the frequency of isolation of *P. cinnamomi* from soil of infested *B. baxteri* and *B. coccinea* (Fig. 5). While frequency of soil isolation of *P. cinnamomi* significantly differed between phosphite treatment for *B. brownii*, frequency of isolation for 2.5 and 5 g phosphite/L treatments was significantly greater than that for the not phosphite treated control.

Artificial infection

The mortality curve for inoculated *B. grandis* not sprayed with phosphite was undulating (Fig. 6). Mortality of plants sprayed with all concentrations of phosphite was significantly lower than those not treated with phosphite (Fig. 6). Due to the large number of zero values for the 2.5 and 10 g phosphite/L treatments, logit-transformation of replicate data could not be done. The apparent mortality rate for the non-phosphite treated control was almost twice that for the 5 g phosphite/L treatment, being 1.1 ± 0.1 and 0.6 ± 0.1/unit/year for 0 and 5 g phosphite/L, respectively. The years to 50% mortality for the non-phosphite treated control was a third of that for the 5 g phosphite/L treatment, being 2.2 ± 0.3 and 6.6 ± 1.6 years for 0 and 5 g phosphite/L, respectively.

Challenge inoculation showed that that spray treatment of 5 g and 10 g phosphite/L were effective in *B. grandis*, 2.2 years after spray (Table 3). There was no significant difference in lesion length, girdling and percentage recovery of *P. cinnamomi* between 2.5 g phosphite/L and the not phosphite treated control (Table 3). Lesion length and girdling in stems sprayed with 5 and 10 g phosphite/L were significantly lower than in stems not sprayed with phosphite. Percentage isolation of *P. cinnamomi* from stems sprayed with 10 g phosphite/L was significantly lower than from stems not sprayed with phosphite.

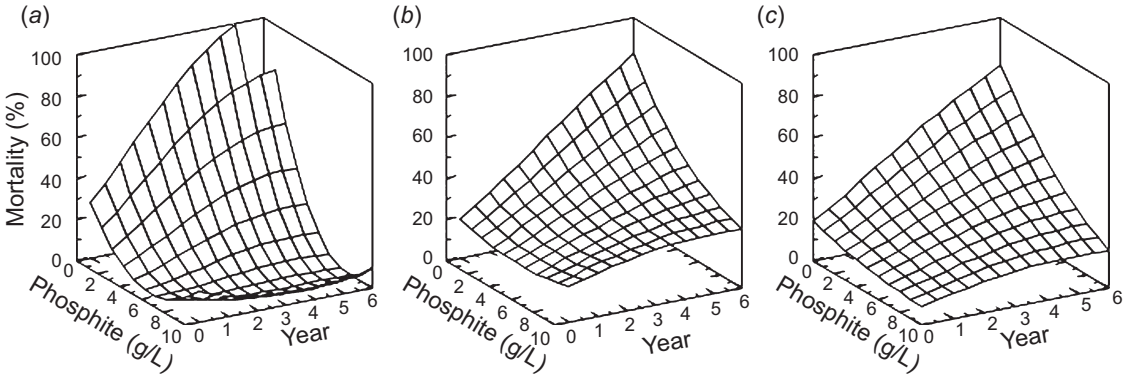

Fig. 4. Change with year after spraying of predicted percent mortality response surfaces following *Phytophthora cinnamomi* infestation of (*a*) *Banksia brownii*, (*b*) *B. baxteri* and (*c*) *B. coccinea* plants with year after foliar spray with 2.5, 5 or 10 g phosphite/L or not sprayed with phosphite. Regression equations: (*a*) *B. brownii*: $\sqrt{}$% Mortality = 5.1485–0.0996 phosphite + 1.5635 year + 0.0099 phosphite^2 − 0.1196 year^2 − 0.0726 phosphite × year ($R^2 = 0.89$); (*b*) *B. baxteri*: $\sqrt{}$% Mortality = 4.3467 − 0.2484 phosphite + 1.0647 year + 0.0234 phosphite^2 − 0.0456 year^2 − 0.0600 phosphite × year ($R^2 = 0.81$); (*c*) *B. coccinea*: $\sqrt{}$% Mortality = 4.3700 − 0.2344 phosphite + 1.0567 year + 0.0101 phosphite^2 − 0.0538 year^2 − 0.0530 phosphite × year ($R^2 = 0.95$).

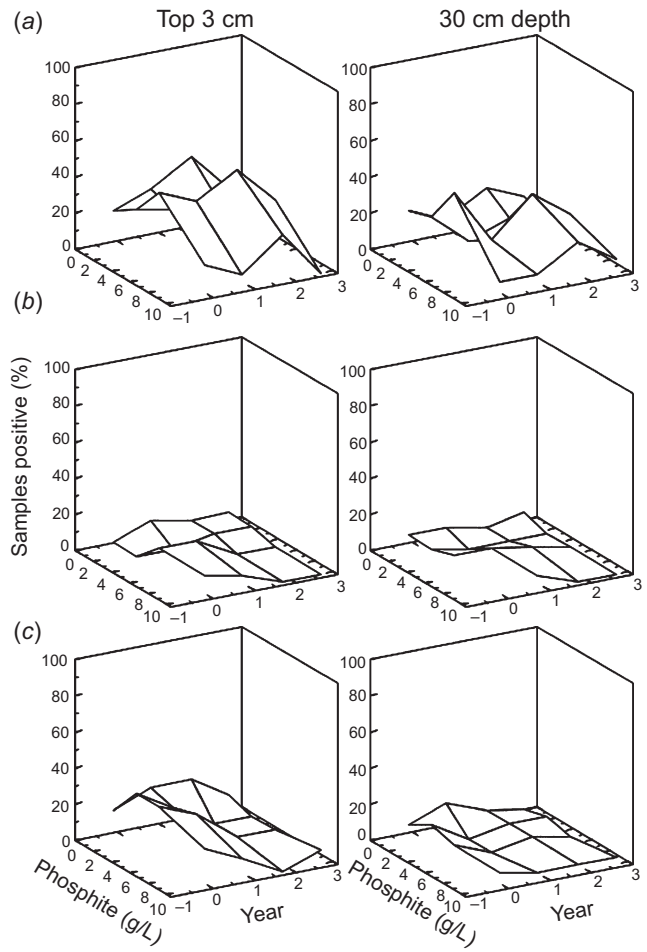

Fig. 5. Changes with year after spraying in the proportion of samples positive for *Phytophthora cinnamomi* in the top 3 cm and at 30 cm below the soil surface assessed once before phosphite application and three times after infested plant communities of (*a*) *Banksia brownii*, (*b*) *B. baxteri* and (*c*) *B. coccinea* were either not sprayed with phosphite or foliar sprayed with 2.5, 5 or 10 g phosphite/L.

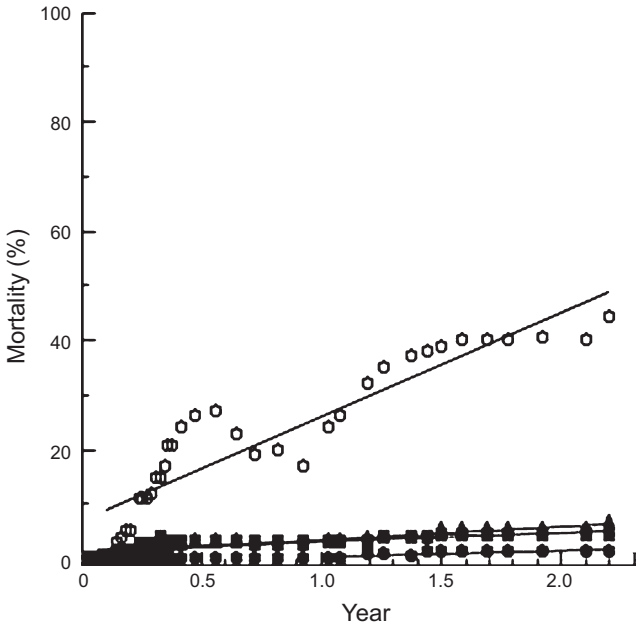

Fig. 6. Changes with year after spraying of percent mortality of *Banksia grandis* plants stem-inoculated with *Phytophthora cinnamomi* that were either not sprayed with phosphite (◯) or foliar sprayed with 2.5 (●), 5 (▲) or 10 (■) g phosphite/L. Continuous lines indicate linear regressions with significant correlation coefficients for the mean values:
0 g phosphite/L: % mortality = 6.18 + 19.50 year ($R^2 = 0.85$);
2.5 g phosphite/L: % mortality = −0.86 + 1.25 year ($R^2 = 0.65$);
5 g phosphite/L: % mortality = 0.99 + 2.52 year ($R^2 = 0.86$);
10 g phosphite/L: % mortality = 1.53 + 1.65 year ($R^2 = 0.66$).

There were significant correlation coefficients for the negative linear relationships between applied phosphite concentration and lesion length and girdling:

$$\text{Lesion length} = 495.96 - 41.64\,\text{applied phosphite concentration}\ (R^2 = 0.97);$$

$$\text{Lesion girdling} = 364.10 - 24.49\,\text{applied phosphite concentration}\ (R^2 = 0.96).$$

Table 3. Mean (± s.e.) lesion length, girdling and percent recovery following challenge inoculation of *Banksia grandis* with *Phytophthora cinnamomi* 2.2 years after foliar spray with phosphite

Phosphite application (g/L)	Lesion Length (mm)	Lesion Girdling (°)	% Recovery
0	479 ± 31	340 ± 9	80 ± 4
2.5	455 ± 30	335 ± 11	87 ± 4
5.0	254 ± 33	258 ± 21	75 ± 5
10.0	88 ± 23	107 ± 16	44 ± 6

Discussion

Analysis of disease progress curves is a powerful tool for understanding epidemic development and process and the effectiveness of disease control treatments (Jeger 2004). Despite this, there has been little determination and analysis of *P. cinnamomi* disease progress curves (Shearer *et al*. 2007). The apparent mortality rates of 0.51–1.1/unit/year found for mortality curves of the not sprayed *Banksia* species are within the range of 0.34–3.46/unit/year found for the rate of mortality of a range plant species susceptible to *P. cinnamomi* in the South-West Botanical Province of Western Australia (Shearer and Fairman 2007; Shearer *et al*. 2007). The apparent mortality rates were reduced to as low as 0.14/unit/year when *B. baxteri* was sprayed with 10 g phosphite/L. No other determination of disease progress of *P. cinnamomi* was found in the literature. In comparison to apparent mortality rates of *Phytophthora* species on agricultural crops, the range of rates found for *P. cinnamomi* in this study were similar to the range of 0.07–3.2/unit/year found by Tooley and Grau (1982) for *P. megasperma* f. sp. *glycinea* on different soybean cultivars. In contrast, much higher apparent mortality rates were found for other *Phytophthora* species. Apparent mortality rates of *P. parasitica* var. *nicotianae* on tobacco varied between 7.3–80.3/unit/year depending on inoculum density and fungicide application (Kannwischer and Mitchell 1978) and those for *P. megasperma* f. sp. *medicaginis* on alfalfa varied between 7.3–131.4/unit/year depending on soil matric potential and inoculum type (Kuan and Erwin 1982). Apparent mortality rates as high as 54.8–160.6/unit/year were found for *P. vignae* on cowpea lines of differing susceptibility to the pathogen (Davis *et al*. 1994). A much greater understanding of *P. cinnamomi* disease progress curves is required for the assessment of changes of disease impact with time and between locations, host susceptibility and determination of the efficacy of control strategies.

The distinct undulating progress of mortality for the *B. grandis* not sprayed with phosphite was probably related to the process of invasion following stem inoculation. There was sufficient stem girdling by *P. cinnamomi* in some plants to cause death of all the leaves in the first summer. However, presumably lesions did not completely girdle all of the stems of plants with dead leaves and these plants resprouted after rains moistened the soil in autumn, resulting in a decline in recorded dead plants. As temperatures increased in spring, the stems of these plants were subsequently completely girdled by *P. cinnamomi* and died with an associated increase in recorded dead plants. Mortality curves for the naturally infested *Banksia* communities were also undulating, but not as pronounced as the artificially stem-inoculated *B. grandis*. Modelling of *P. infestans* epidemics suggest that disease severity curves can increase in an undulating manner (Zadoks 1971). A much greater understanding of *P. cinnamomi* disease progress curves is required to determine whether an undulating increase is a common occurrence.

In the South-West Botanical Province of Western Australia the population dynamics of *P. cinnamomi* have been determined for soils of *E. marginata* forest (Shearer and Shea 1987), but not for other bioregions of the province. Little is known of the dynamics of the pathogen in soils of the Esperance Plains bioregion where the *B. brownii*, *B. baxteri* and *B. coccinea* communities occurred. Greater isolation of *P. cinnamomi* from the top 3 cm of soil than 30 cm below the soil surface in infested *B. brownii* and *B. coccinea* was opposite to that found in *E. marginata* forest. Generally the pathogen was more frequently isolated from forest soil between 10–80 cm below the soil surface than from the top 10 cm (Shearer and Shea 1987). This difference could be due to several factors including sampling time, soil microbial composition, soil properties and environment. How such factors interact with pathogen dynamics in south coast soils is not known. A much greater understanding of *P. cinnamomi* population dynamics in soils of the Esperance Plains bioregion of the South-West Botanical Province of Western Australia is required in order to determine changes of disease impact with time and between locations and the efficacy of control strategies within the region.

Phosphite had no significant effect on frequency of isolation of *P. cinnamomi* from soil of foliar sprayed plants in the infested *Banksia* communities. This result is similar to that found in agriculture where neither foliar spray (Darvas 1983; Matheron *et al*. 1997) nor injection (Roux *et al*. 1991) with fosetyl-Al (an ethyl phosphonate) reduced inoculum of *Phytophthora* species in soil under treated plants compared to not treated plants. However, a foliar drench of fosetyl-Al significantly reduced *P. cinnamomi* chlamydospore numbers in soil under treated pineapple (Allen *et al*. 1980) and a root drench or foliar spray of phosphite significantly reduced soil inoculum levels of the pathogen in pot trials (Ali and Guest 1998; Ali *et al*. 1999; Swart and Denman 2000; Aryantha and Guest 2004). Sporangia production of *Phytophthora* species was reduced, but not prevented by phosphite application (Wilkinson *et al*. 2001*b*). As *P. cinnamomi* is a poor saprophytic coloniser and the zoospores and chlamydospores live for a limited time (Shearer and Smith 2000; McCarren *et al*. 2005), pathogen survival in phosphite treated areas will be determined by the ability of the pathogen to infect healthy tissue protected by phosphite. The test of the effect of phosphite on inoculum survival in the sprayed plots in the infested *Banksia* communities was probably severe, as foliar sprayed plants ended up as islands of live plants surrounded by dead and dying plants. Thus, the plot areas where plants had been foliar-sprayed by phosphite were surrounded by a constant supply of inoculum, which would not have occurred if the whole community was sprayed. Possibly a longer period than the 2.7 years monitored may have been needed to determine the effect of phosphite on soil population levels in sprayed plots. Further research is required to determine whether long-term phosphite application to infested plant communities may be effective in reducing soil inoculum levels of *P. cinnamomi*.

Foliar spray of 10 g phosphite/L caused significant stunting of *B. coccinea* similar to the effects of growth regulator imbalance. Growth abnormalities that included rosetting and stunting, increased with the concentration of phosphite in low-volume spray foliar application and the highest incidence occurred in Proteaceae on infertile leached deep sands (Barrett 2003). However, at the recommended foliar application rate, the majority of plant species had mild symptoms and recovered after 2 years (Barrett 2003). Growth retardation following phosphite application has been found in a range of agriculture crops such as mandarins, maize, brassica and onion (Walker 1989; Wellings *et al*. 1990; Seymour *et al*. 1994; Carswell *et al*. 1996; Sukarno *et al*. 1998). Increasing concentration of injected phosphite stresses *B. coccinea*, resulting in decreased inhibition of *P. cinnamomi* in treated trees (Shearer *et al*. 2006). Stress can change levels of plant growth regulators and the plant's sensitivity to them (Davies *et al*. 1986; Yordanov 1995). Plant growth regulator responses of stressed plants are likely to be complex (Cramer 2002). That auxin growth regulator genes in *Arabidopsis* were induced in the presence of growth-inhibitory phosphite concentrations indicated that the expression of phosphite was selective and not caused by a possible general cellular toxicity of phosphite (Ticconi *et al*. 2001). Priority must be given to determining phosphite mediated changes to the biochemistry of treated plants in order to elucidate the mechanisms of action of phosphite with respect to phytotoxic reactions and control of *Phytophthora* species.

The organo-silicone adjuvant Pulse enhances spray coverage and uptake into foliage (Knoche 1994). Increased silicon absorption can protect a range of plant species against fungal disease, although the extent of the prophylactic effect may vary between hosts and study (Bélanger *et al*. 1995). A foliar spray of sodium silicate resulted in significant reduction in lesion lengths of *P. cinnamomi* in stems of *B. brownii* and *B. coccinea*, but not *B. speciosa* (Smith 1994). Any effect of silicon from Pulse on disease development would not be a factor in this study, as the plots sprayed and not sprayed with phosphite were all sprayed with Pulse. In addition, other trials with *B. attenuata*, *B. baxteri* and *Lambertia inermis* var. *inermis* have shown that there was no statistical difference in lesion lengths of *P. cinnamomi* in stems sprayed and not sprayed with Pulse (C. E. Crane and B. L. Shearer, unpubl. data).

The mortality curves of *B. brownii*, *B. baxteri* and *B. coccinea* and challenge inoculation of *B. grandis* suggests that phosphite effectiveness against *P. cinnamomi* lasts at least 2 years following high-volume foliar spray of the four *Banksia* species tested. This period of phosphite effectiveness following foliar spray is similar to the 0.5–2 years of effectiveness found for a range of native plant species (Aberton *et al*. 1999; Tynan *et al*. 2001; Wilkinson *et al*. 2001*a*; Barrett 2003) but much longer than the 0.06–0.9 years found for a range of agricultural plant species (Davis 1981; Wicks and Hall 1988, 1990; Lim *et al*. 1990). Shearer and Fairman (2007) concluded that differences in longevity of phosphite effectiveness between agricultural and native plant species could be due to high phosphate concentrations imposed in agriculture compared to the much lower phosphate levels occurring in native communities and by differences in the persistence of the compound in agriculture crops compared to that in native flora. Various adaptations of native plant species to summer drought and infertile soils have resulted in slow growth rates and maximisation of residence times of nutrients through longer lived organs and higher resorption rates from senescing organs (Specht and Groves 1966; Chapin 1980; Grierson and Adams 1999; Aerts and Chapin 2000; Reich *et al*. 2003; Wright and Westoby 2003), characteristics that would be expected to maximise retention of phosphite in native plants.

The persistence of phosphite effectiveness of at least 2 years against *P. cinnamomi* following high-volume foliar spray is half the 4 years of effectiveness found following stem injection of native plant species (Shearer *et al*. 2004, 2006; Shearer and Fairman 2007). There are few reports of the influence of different application methods on phosphite effectiveness and levels in the plant (Shearer *et al*. 2004). Although phosphite levels in roots of 4-year-old citrus trees were greater following foliar spray than stem injection, the fungicide persisted longer in roots following stem injection than foliar spray (Schutte *et al*. 1991). Different methods of phosphite application should be compared in order to determine the effects of application method on phosphite uptake, redistribution and persistence within the plant and duration of effectiveness against *P. cinnamomi*.

Demonstration that high-volume foliar application reduced mortality of plant species threatened by *P. cinnamomi* infection resulted in the development of aerial low-volume foliar application (Komorek *et al*. 1997). Currently foliar phosphite application is one of the main strategies protecting rare and endangered flora, such as *B. brownii*, from *P. cinnamomi* infection (Barrett 2005; Shearer *et al*. 2007). Current application rates maintain a balance between effective control of *P. cinnamomi* with minimisation of phytotoxic reactions following phosphite application to target plant species. For example, because of growth abnormalities in *B. coccinea*, high-volume application rates >5 g phosphite/L are not recommended. Determination of the variation of phosphite effectiveness against *P. cinnamomi* and phytotoxic responses between plant species will assist prescription optimisation for the most effective protection of threatened flora.

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