Novel phosphite and nutrient application to control *Phytophthora cinnamomi* disease

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Abstract

Systemic treatment of stems with injections of phosphite liquid and novel soluble capsule implants of phosphite, PHOSCAP® (phosphorous, potassium, iron, manganese, zinc, boron, copper, magnesium and molybdenum) and MEDICAP MD® (nitrogen, phosphorous, potassium, iron, manganese, and zinc), were applied to *Banksia grandis* and *Eucalyptus marginata* trees to control *Phytophthora cinnamomi*. Four weeks after treatment application, excised branches were under-bark inoculated with *P. cinnamomi*. In *B. grandis*, phosphite implants and liquid injections significantly reduced lesion length compared to the control, and MEDICAP MD® implants; however, there was no significant difference in lesion length between trees treated with phosphite implants and liquid injections and PHOSCAP implants. In *E. marginata*, phosphite implants and liquid injections significantly reduced lesion length compared to the control, PHOSCAP® and MEDICAP MD®

implants. In *B. grandis* and *E. marginata*, PHOSCAP® and MEDICAP MD® implants reduced the average lesion length compared to the control; however, the interactions were not significant. Results show that both liquid phosphite injections and novel phosphite implants are effective at controlling lesion extension in *B. grandis* and *E. marginata*, caused by *P. cinnamomi*. Further work is required to determine if nutrient application reduces *Phytophthora* disease through improving plant health.

Keywords: Phosphite implant; Phosphoric acid; *Eucalyptus marginata; Banksia grandis;* Natural Ecosystems

Introduction

A range of tools are required to control devastating plant diseases within natural ecosystems and agriculture, caused by *Phytophthora* species worldwide. To help manage *Phytophthora* diseases, solutions of phosphonic acid, active ingredient phosphite, have been routinely applied through liquid injections and foliar sprays (Hardy et al. 2001). Stem injections of liquid phosphite have been shown to protect *B. grandis* and *E. marginata* from *P. cinnamomi* for at least four years (Shearer and Fairman 2007). However, stem injections or foliar spray of phosphite may be restrictive and laborious, as some specialized equipment and training is required to apply the correct concentration and apply the liquid chemical. More recently, soluble, slow-release implants of phosphite have been developed that can be inserted into the stems, without the need to mix chemicals or use injection equipment. This technique still requires drilling into trees and may not be feasible for rapid widespread use in natural ecosystems, where the greatest threat to biodiversity lies. However, it is feasible for selective use on larger stemmed shrubs and trees in small reserves, private properties and horticultural situations.

South-western Australia is an ancient, semi-arid to mediterranean land with a diverse native flora, long adapted to the nutrient poor soils which require the application of considerable quantities of

fertilizers and trace elements for the economic cultivation of crops and pasture (Hodgkin and Hamilton 1993). Host micronutrient deficiencies have been associated with reduced disease resistance, known to involve diverse biochemical systems (Nelson 1978). Native plants, growing in nutrient low ecosystems may be particularly prone to reduced disease resistance resulting from disturbances, including fungal disease, as these plants may already function near thresholds where nutrient availability limits a range of biochemical process. Nutrient amendments may therefore be specifically valuable at improving disease resistance for plants growing naturally on nutrient low sites.

It is possible that disturbance may lead to nutrient imbalances in mature trees, increasing the susceptibility to pests and diseases (Van Miegroet and Johnson 2009). Systemic nutrient implants including PHOSCAP® and MEDICAP® (Creative Sales, Inc., Fremont, Nebraska, United States of America) treatments and injections have been effectively used to correct nutrient deficiencies in ornamental and horticultural plants including: *Quercus* species (oak), *Prunus avium* (flowering cherry) and *Acer* species (maple) (Smith 1978; Harrell et al.1984), *Pinus* species (pine), *Liquidambar* species (sweet gum), *Magnolia* species, *Photinia villosa* (oriental photinia) (Smith 1978) and *Carya illinoinensis* (pecan) (Worley and Littrell 1978; Worley et al.1980). Nutrient implants have been successfully used in native vegetation within Western Australia to help manage disease in the *Eucalyptus gomphocephala* and *Banksia* ecosystems (Scott et al.2013).

This study aimed to determine how novel soluble implants of phosphite, phosphate plus combined nutrients and combined nutrients applied alone compared to liquid phosphite injections for the control of disease in *B. grandis* and *E. marginata* caused by *P. cinnamomi*.

Method

Trees were located in *E. marginata* (jarrah) forest (31.931090 °S, 116.183202 °S) in Mundaring National Park, approximately 31 km east of Perth, Western Australia. The trial site was approximately 0.25 ha in size and located about 100 m from an active *P. cinnamomi* front. The region has a

Mediterranean climate and receives approximately 1091.6 mm rainfall annually, mainly over winter (June–August), and a mean maximum/minimum temperature of 22.5/11.2 °C (1994–2012), recorded at Bickley approximately 9.5 km from the trial site (BoM 2012).

In March (autumn) 2009, ten trees each of *B. grandis* and *E. marginata* were treated with one of five treatments: (1) phosphite liquid, (2) phosphite implants (3) PHOSCAP®, (4) MEDICAP MD®, and (5) a control. *Banksia grandis* trees, with circumference over bark from 80 to 270 cm [mean (± SEM) of 113.2 ± 6.3 cm] and *E. marginata* trees, with diameter at breast (1.5 m) height (DBH) from 81 to 270 cm [mean (±SEM) of 165.9 ± 6.6 cm], were selected at random. Treatments were allocated to individual trees by ranking trees in order of DBH, and evenly allocating treatments across the size range.

Treatment application

A 75 g phosphite/L aqueous solution was made from a 200 g/L commercial formulation [Fosject-200, UIM Agrochemicals (Australia) Pty Ltd (Rocklea, Queensland, Australia) containing 200 g $H_2(PO_3H)$/L present as mono-di potassium phosphite, adjusted to pH 5.7–6.0], diluted with deionized water. Phosphite was injected at 1 mL/cm of stem circumference, equivalent to 750 mg phosphite/10 cm trunk circumference at breast height or 1.5 m above ground level. Holes were drilled through the outer bark layer into the sapwood at 20 cm intervals with a 6.5 mm drill bit and the phosphite solutions were injected using 20 mL spring-loaded tree syringes that lock tightly into the trees (Chemjet Pty Ltd, Bongaree, Queensland, Australia) (Shearer et al. 2006).

Soluble powder implants of phosphite, PHOSCAP® and MEDICAP MD® (Table 1), were applied in accordance with the manufacturer's instructions. Implants consist of gelatine capsule containing the relevant compound in a powdered or crystalline form housed within a rigid polyurethane casing containing caps to allow sap to flow past and dissolved the gelatine capsule and its contents. These implants, in contrast to liquid injections, allows the compound to slowly dissolve within the sapwood as the trees transpire, providing a more passive uptake of the active ingredient when compared with the pressurised liquid injection system. Implants were applied as close as possible to the

recommended height of between 0.5 and 1 m above ground level, at 10 cm intervals. Implants were 0.95 cm in diameter and 3.2 cm in length. Holes were drilled with a 0.95 cm bit, approximately 4 cm into the cambium. Drill shavings were removed and the implants were manually inserted, until they were flush with the cambium. Control implants, comprised solely of the outer casing without any active ingredients, were applied as per the implants of phosphite, PHOSCAP® and MEDICAP MD®.

Excised branches and inoculation

Phytophthora cinnamomi isolate MP94-48 (Murdoch University *Phytophthora* collection) was passaged through a Green Granny smith apple (*Malus domestica* × *M. sylvestris*) 8 weeks prior to inoculation, to ensure that the isolate had not lost its pathogenicity as a result of prolonged subculturing (Erwin and Ribeiro 1996).

Excised stems were processed using modifications of Hüberli et al. (2001). Four weeks after treatment application, a green side branch approximately 38 cm × 20–40 mm diameter of *B. grandis*, and 38 cm × 30–80 mm diameter of *E. marginata*, was removed from each treated tree (Shearer et al.1987). All side shoots and leaves were immediately removed in the field, and the side branch was transported to the laboratory in moist hessian bags. In the laboratory, branches were surface-sterilised with 70 % ethanol and the exposed ends were immediately dipped into melted wax in order to minimise desiccation (Hüberli et al. 2002). A sterile scalpel was used to cut a bark-flap, about 15 mm long and 10 mm wide, approximately half-way up the stem and along internodes, through the epidermis to the phloem. A 5 mm diameter agar disc, cut from the margin of a 3-day-old culture growing on V8 agar [100 mL/L V8 vegetable juice (Campbell's®), 900 mL/L demineralised water, 3 g/L CaCO3 and 15 g A Grade Agar (Becton, Dickinson and Company, Sparks, USA)], was inserted mycelium-side-down under the flap. The flap was closed and the wound was sealed with Parafilm (American National Can, Chicago, USA) tape and aluminium foil (Shearer et al.1988; O'Gara et al.1996). Plugs of non-colonised V8 agar were used for the control inoculations. Stems were incubated at 24 °C in the dark in disinfected plastic trays lined with moist paper towels, and sealed in plastic bags.

Lesion formation was recorded 6 days after inoculation. Previous research has shown that the rate of lesion extension in excised stems of *E. marginata* inoculated with *P. cinnamomi* increases after 8 days, probably as a result of stem senescence (Hüberli et al.2001). Colonisation above and below any visible lesion was assessed by plating 1 cm stem sections from 5 cm above and below the visible lesion onto PARPNH agar [V8 vegetable juice (Campbell's®) 100 mL/L, 20 g/L A Grade Agar (Becton, Dickinson and Company, Sparks, USA), $CaCO_3$ 3 g/L, pimaricin l0 mg/L, ampicillin 200 mg/L, rifampicin 10 mg/L, pentachloronitrobenzene (PCNB) 25 mg/L, nystatin 50 mg/L and hymexazol 50 mg/L] modified from (Tsao 1983), giving a total of 10 sections per plant. The initial inoculation section was also plated onto PARPNH to confirm infection.

Statistical analysis

Significance was determined at $P \leq 0.05$. Assumptions of normality were checked by plotting residuals according to Clarke and Warwick (2001). The significance of injection and implant treatments on lesion length were determined using separate statistical analysis for *B. grandis* and *E. marginata*. A one-way ANOVA was used to test for significant differences between the means of each treatment. Where treatments were significant, *post hoc* Fisher LSD tests were used to identify significantly different factor levels (Day and Quinn 1989). Analyses were carried out in Statistica® software package Version 5 (Statsoft 1999).

Results

Phytophthora cinnamomi was reisolated from the original inoculation point from all excised stems. In B. grandis, phosphite implants and liquid injections significantly reduced lesion length compared to the control and MEDICAP MD® implants; however, there was no significant difference between lesion length in trees treated with phosphite implants and liquid injections and PHOSCAP® (Fig. 1a). In E. marginata, phosphite implants and liquid injections significantly reduced lesion length compared to the control and MEDICAP MD® and PHOSCAP® implants (Fig. 1b). There was no significant difference in lesion length between trees treated with phosphite implants and liquid phosphite

injections in both B. grandis and E. marginata. In both tree species there was no significant difference in lesion length between the control and MEDICAP MD® and PHOSCAP® implants, although treatment with MEDICAP MD® and PHOSCAP® reduced lesion length compared to the control.

Discussion

Results confirm that both phosphite implants and liquid phosphite injections significantly reduce lesion length caused by *P. cinnamomi* in under bark inoculated excised branches. Further work is required to understand how both phosphite and nutrient amendments impact disease severity. Phosphite is known to control *Phytophthora* associated disease by inducing a strong and rapid host defence response and by directly acting on the pathogen (Hardy et al.2001). Nutrient applications may have increased crown health by ameliorating an underlying nutrient deficiency, increasing resistance to a decline pressure or disease, improving symptoms of decline, or combinations of these factors.

The novel phosphite implants were an effective delivery mechanism for applying phosphite in a sufficient concentration to control *P. cinnamomi*. Phosphite implants would likely control *Phytophthora* diseases in species where phosphite liquid injections have been effective. Application rates may be manipulated by varying the spacing between implants. However, to prescribe suitable application rates, further work is required to determine how much phosphite within the implants is transferred to treated plants. To calculate the efficiency of phosphite uptake from soluble implants, phosphite concentration within treated plants may be determined using gas chromatography (Barrett et al. 2004).

Phytotoxic symptoms of leaf curl, leaf drop and stunted leaves have been observed in *B. grandis* and *E. marginata*, following liquid phosphite injections at concentrations of 50, 100 and 200 g phosphite/L and application rates of 1 and 2 mL/cm of stem circumference (Shearer et al. 2006). The phosphite implant treatments used in this study were equivalent to 58 g phosphite/L at application rates of 1 mL/cm, and are therefore unlikely to cause lasting damage. In addition, the rate of uptake of the active ingredient is likely to be slower than in pressurised liquid injection systems,

thereby reducing the potential for phytotoxicity. It is possible that phosphite implants could cause phytotoxicity if the implants are applied at higher rates or to more sensitive plants.

MEDICAP MD® and PHOSCAP® implants reduced the average lesion extension in both *B. grandis* and *E. marginata*, although the interactions were not significant. Both of these treatments contain a range of nutrients, with MEDICAP MD® designed as a broad spectrum nutrition treatment, containing N, P, K plus a range of trace elements such as Fe, Mn and Zn, and PHOSCAP® implants designed as a treatment containing high levels of P and K and additional trace elements such as Cu and Bo, which are important for the production of cellulose, lignin, and a range of proteins (Snowdon 2000; Dell et al. 2001). Nutrient application may improve resistance to pests and diseases, especially in plants where nutrient availability may limit growth. Nutrient amendments may reduce disease expression by increasing resistance, or increasing tolerance to pathogens (Graham and Webb 1991). For example, manganese is required for the activity of glycoproteins (lectin), which are associated with potato resistance to *Phytophthora infestans* (late blight) (Garas and Kuc 1981). Boron application may reduce disease in deficient trees by improving chemical defences and improving cell wall structure and membrane stability (Dordas 2008; Lehto et al.2010). Soil micro-nutrient infertility has also been linked to increased *P. cinnamomi* disease severity of on some sites (Shearer and Crane 2011). However, the application of some nutrients including N, P, K, Mg, Zn and Cu, have been shown to decrease and increase damage caused by different pathogens in different plants To sustainably manage any disease, the etiologies of any nutrient deficiencies need to be accurately diagnosed and a holistic approach taken to correct the disorder.

Inoculation of excised stems and roots has been used to determine the susceptibility of a range of host species to *P. cinnamomi* (Tippett et al.1985; Shearer et al.1987; Hüberli et al. 2001) *P. alni*, *P. cambivora and* other *Phytophthora* species (Brasier and Kirk 2001). While convenient and relatively quick, there is a poor correlation between measurements from excised tissue and those from natural environments (Tippett et al.1985; Hüberli et al.2001). However, inoculation of excised stems provides sufficient resolution to confirm if phosphite implants provide comparable protection to liquid phosphite injections.

Stem injections and implants result in varying degrees of damage to treated plants (Costonis 1981), which may negatively affect plant health by causing toxicity, reducing structural integrity, impeding vascular activity and growth and facilitating the entry of pests and diseases. The benefits of treatment may significantly outweigh the costs of not treating disease-affected trees. The amount of damage caused by liquid injections or implants depends on a number of factors, including the number of injections/implants, size of the injection wound, vigour of the treated plants, species, time of year, mode of delivery, and chemical formulation (Costonis 1980; Doccola et al. 2007). Systemic fungicide injections to control Dutch elm disease have been shown to cause more damage than benefit by causing wound necrosis induced by chemical toxicity (Anderson et al.1979) with drill wounds allowing the entry of bacterial wilts (Campana et al.1979). In contrast, a more recent study measuring the tree wound response following systemic insecticide injection treatment showed all healthy, treated trees successfully compartmentalized injection wounds with no evidence of decay, infection or structural damage (Doccola et al.2011). The costs and benefits of any injection or implant treatment program must be considered prior to application.

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Table 1 Composition of implants of phosphite, PHOSCAP® and MEDICAP® (Creative Sales, Inc., Fremont, Nebraska, United States of America)

Implants	Weight per capsule	Capsule constituents	Composition % by weight	Dose (mg/10 cm trunk circumference)
Phosphite implants	1.0 g	Phosphite	58	580
		Phosphate	4	40
		Soluble potash	38	380
PHOSCAP®	0.8 g	Phosphate	50	400
		Soluble potash	30	240
		Magnesium	0.06	0.48
		Boron	0.02	0.16
		Copper	0.05	0.4
		Iron	0.1	0.8
		Magnesium	0.05	0.4
		Molybdenum	0.0005	0.004
		Zinc	0.05	0.4
MEDICAP MD®	0.8 g	Total nitrogen	12	108
		Ammoniacal nitrogen	1	9
		Nitrate nitrogen	1.5	14
		Urea nitrogen	9.5	86
		Phosphate	4	36
		Soluble potash	4	36
		Iron	4	36
		Manganese	4	36
		Zinc	4	36

Fig. 1 Mean lesion length (± standard error) of *Phytophthora cinnamomi* in under-bark inoculated excised branches of (**a**) *Banksia grandis* and (**b**) *Eucalyptus marginata* after treatment with: blank implants (Control); MEDICAP MD® implants (MED MD); PHOSCAP® implants (PHOS); phosphite implants (Phi imp) and phosphite liquid injections at 75 g phosphite/L (Phi liq). Statistics are for one-way ANOVA. ** $P \leq 0.01$, *** $P \leq 0.001$. *Small letters* denote the results of the *post hoc* test (Fisher LSD) where *bars with the same letters* are not significantly ($P \leq 0.05$) different