THE POTENTIAL OF THE FUNGICIDE PHOSPHITE TO CONTROL *PHYTOPHTHORA CINNAMOMI* IN NATIVE PLANT COMMUNITIES ASSOCIATED WITH MINING

Results of research carried out as MERIWA Project No. M280 at the School of Biological Sciences, Murdoch University

by

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December, 2000

Distributed by: MERIWA
Mineral House
100 Plain Street
EAST PERTH WA 6004

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ISBN 0 7309 8525 3
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Acknowledgments

This work was funded by Minerals and Energy Research Institute of Western Australia, Australian Research Council, Alcoa World Alumina Australia, Iluka Resources, Tiwest Joint Venture, Worsley Alumina and Sons of Gwallia.

We would like to take the opportunity to thank the following people who were associated with the activities of this project.

1. Jason Maroudas; Chemist.
2. Chris Loane; Chemist.
3. Doug Clarke for assistance with developing the HPIC protocols.
4. Matt Williams from CALMScience for analysis of much of the data.
5. Mike Calver for his help with experimental design.
6. Dr. Keith McDougall; Temporary Research Associate.
7. Dr. Greg Roos for assistance with developing the HPIC protocols.
8. John Collins, Tracie Easter and Libby Burgess for their long hours and invaluable technical assistance.
9. Rosalyn Pilbeam; PhD student.
10. Meredith Fairbanks; PhD student.
11. Kay Howard; PhD student.

In addition, we would like to thank the following who assisted in the establishment and harvest of field and glasshouse trials: Diane White, Graham Blackwell, Daniel Hüberli, Sussan Nawell, Rebecca Hamilton, Sarah Collins, Meredith Fairbanks, Chris Dunne, Tania Jackson, Ryan Munro, Mark Farbey, Aaron Maxwell, Karen Paton, Troy Easter, Anne Lucas, Valerie Ee, Khaled El-Tarabily, Catherine Chamberlain, Adam Pratt, Kay Howard, Paco Tovar, Jason Schmidberger, Nola D’Souza, Julie Crosby, Fiona Sanger and Robin France.
Executive Summary

This project aimed to assess whether foliar application of phosphite is a practicable, economic and effective way of controlling *Phytophthora cinnamomi* in native plant communities. Table 1 addresses the objectives, associated activities and outcomes. A list of key recommendations and suggested future research activities follows Table 1.

Table 1. Project objectives, the activities undertaken to meet the objectives and the outcomes of the project.

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• Hygiene measures must be maintained in areas treated with phosphite as *P. cinnamomi* is not killed *in planta* and is capable of producing zoospores. |
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<td>• Benefits of phosphite application in spring and autumn varied between plant species and plant communities.</td>
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- Effect of phosphite on phytotoxicity.
- 5 g phosphite/L is the recommended rate of application as the mild phytotoxicity it causes is not considered an issue for conservation.
- 10 and 20 g phosphite/L caused severe phytotoxicity symptoms in a wide range of plant species.

- Effects of phosphite on pollen viability, seed production and viability (associated PhD project).
- Pollen viability in some species was reduced for more than one year.
- Seed germination was not affected in *Dryandra sessilis*, *Lasiopetalum floribundum* and *Trymalium ledifolium* but germination was affected in *Pterocheata paniculata*.

### E. To determine if phosphite is detrimental to ectomycorrhiza

- Effects of phosphite on beneficial fungi associated with roots of native plants (associated PhD project).
- Three species of eucalypt ectomycorrhizal fungi were not adversely affected by phosphite.
RECOMMENDATIONS

Our research has shown that the application of phosphite has a narrower range of uses than originally envisioned. Phosphite does not kill *P. cinnamomi* or stop zoospore production from diseased tissues when applied at the recommended concentrations. Its effectiveness and persistence varies with season of application and species treated. Therefore, we recommend that phosphite application be closely targeted to operations in areas where the spread of infection would have high impact. Outlined below are a number of operational and research recommendations that have been derived from our research activities.

Operational recommendations

1. **Phosphite should be sprayed on the foliage as a 5 g phosphite/L solution.** This is the optimum rate that increases resistance without causing severe phytotoxicity.

2. **Apply phosphite every year to maintain efficacy.** Our studies have shown that the efficacy of phosphite declines markedly between 6-12 months after application.

3. **Treat vegetation in spring, or in autumn following the break of season.** While it may be effective to spray outside these times, factors such as water deficit and plant stress may adversely effect uptake of phosphite. Application of phosphite in winter may be effective if it does not rain for more than 7 hours after application. However, more research is required to determine whether the low temperatures in winter affects uptake and transport of the chemical within the plant.

4. **Phosphite application is not an alternative to dieback management hygiene procedures.** Hygiene measures must be maintained in areas that have been treated with phosphite.

5. **The efficacy of phosphite must be monitored over time by mapping disease fronts and plant deaths before and after spray application.** Since the effectiveness of phosphite varies between plant species and with season of application, it is important to monitor disease fronts to ensure that the chemical is effective.

6. **There may be situations where it is acceptable to use 20g phosphite/L or higher levels although this will have severe phytotoxic effects on the vegetation.** For example, in small infestations where the risk of *P. cinnamomi* spreading into the surrounding vegetation is large. This concentration should increase the efficacy of phosphite.

RECOMMENDED RESEARCH AND OPERATIONAL TRIALS

We believe that there would be great benefit from undertaking the following Research and Operational trials.

Research Trials.

The following research trials are recommended, as they will improve our understanding of phosphite and its effective use in native plant communities.

1. **To determine the mode of action of phosphite in planta.** This will help explain why phosphite is more effective in plants of the same species growing in different environmental conditions.
(glasshouse versus field trial results) or in different plant species and why phosphite is effective for longer when it is applied using trunk injection rather than spray application. Such knowledge will result in the more effective use of the fungicide in the field.

2. **To determine the effect of repeated applications of phosphite on the capacity of annual and perennial plant species to produce viable seed in the medium to long term, to ascertain if reproductive capacity is reduced.** Since our findings indicate that (a) phosphite should applied on an annual basis, and (b) reproductive fitness is reduced in some plant species, it is necessary to ascertain if repeated applications of phosphite will have adverse effects on the reproductive capacity of different plant communities.

3. **To screen a range of adjuvants in conjunction with phosphite to determine whether phosphite uptake, effectiveness and persistence can be increased in native plant communities.** Our studies only used the sticking agent Synertrol in conjunction with phosphite. It is possible that other adjuvants may (a) increase phosphite uptake, (b) reduce phytotoxic effects of phosphite and thereby allow higher concentrations to be applied, and (c) increase the persistence of phosphite, thereby increasing its effectiveness.

4. **To understand the mechanisms of phosphite uptake by plants with and without adjuvants.** An understanding of how phosphite is taken up and translocated throughout different plant species may provide us with opportunities to increase the ability of plants to absorb phosphite and consequently enhance its effectiveness.

5. **To determine the cause(s) of phytotoxicity.** If the levels of phytotoxicity can be decreased then it may be possible to apply higher concentrations of phosphite.

6. **To ascertain if phytotoxic concentrations of phosphite will kill *P. cinnamomi* in planta.** Under certain conditions extreme phytotoxic symptoms may be acceptable if *P. cinnamomi* is killed.

7. **To determine the effects of temperature and/or moisture stress on phosphite efficacy in planta.** Temperature and moisture are known to be important factors in disease development. However, how these factors interact with phosphite and its effects on *P. cinnamomi* are not known.

8. **To determine if continued application of phosphite will select for phosphite tolerant *P. cinnamomi* isolates.** If phosphite tolerant isolates develop after repeated use of phosphite it will be necessary to be more cautious when using the chemical.
Operational trials

1. **Evaluate the use of phytotoxic concentrations of phosphite to reduce the spread of *Phytophthora cinnamomi* from spot infections.** At the recommended rate of 5g phosphite/L, *P. cinnamomi* is contained in plants for limited periods of time, but not killed and consequently it can re-establish itself once the effects of phosphite have worn off. In addition, phosphite does not stop *P. cinnamomi* sporulating from infected tissues. Therefore, it would be appropriate to trial phytotoxic concentrations of phosphite to determine if these concentrations (a) can kill the pathogen *in planta*, (b) prevent sporulation from contained lesions, and (c) if plants can recover from severe phosphite induced phytotoxicity.

Determine the effectiveness and persistence of 5g phosphite/L to slow or stop the spread of *P. cinnamomi* along 'dieback fronts'. **All our research concentrated on under bark inoculations and the subsequent colonisation of phosphite treated or non-treated tissues by the pathogen. It is now necessary to ascertain if phosphite will stop or slow the rate of spread of *P. cinnamomi* under natural conditions.**
Chapter 1

General Introduction

Phytophthora cinnamomi is a major pathogen of native plant communities in Western Australia (Dell et al. 1989; Shearer et al. 1989; Wills 1993). It affects approximately 14% of the northern jarrah (Eucalyptus marginata) forest in Western Australia (Davison et al. 1989), and over 2000 of the 9000 plant species in the Banksia woodlands and heathlands of the south-west of Western Australia may be susceptible (Wills 1993). Mining and timber harvesting are major activities in areas where dieback disease is prevalent. As part of these operations native plant communities are mapped for the presence of P. cinnamomi. Mapping of the jarrah forest has identified both large continuous and ‘spot’ infestations (<0.3 ha) of disease caused by P. cinnamomi. These spot infestations can be near the top of a slope. The risk of P. cinnamomi spreading to the healthy, uninfected forest down slope is high because the pathogen is microscopic and readily spreads in water.

The presence of P. cinnamomi in and around mining operations in Western Australia increases the financial cost for most mines. A wide range of Phytophthora control measures have been developed to minimise the spread of the pathogen and reduce its impact (Colquhoun et al. 1994) which are a major cost to mining companies. In bauxite mining the premining operations associated with ‘spot infestations’ are also complex and expensive. In addition, monitoring of Phytophthora free forest before and after bauxite mining has found new spot infestations adjacent to rehabilitated mined areas (Crosbie et al. 1999). The development of a method to contain or eradicate P. cinnamomi would be a great financial advantage to mining companies.

The impact of the presence of P. cinnamomi in and around mining operations is not only financial, its presence has the potential to adversely affect three important objectives for the environmental management of mining leases,

- protection of the vegetation in and around mined areas,
- re-establishment of key plant species in rehabilitated mined areas, and
- achievement of high species richness in rehabilitated mined areas (Colquhoun et al. 1994).

Although fungicides have been widely used in agronomic situations, they have rarely been used in native plant communities due to factors such as high costs of the chemicals, unknown phytotoxic responses and difficulties associated with applying the chemical. Recently, research has shown that the use of neutralised phosphorous acid (phosphite), has considerable value in the conservation of rare and endangered plant species in the south-west of Western Australia (Shearer et al. 1991). This fungicide is inexpensive, of low toxicity to plants and animals and has high mobility within plants. In the past there was no potential treatment to eradicate P. cinnamomi in the soil or even reduce the risk of its spread. However, phosphite offers the opportunity to protect the plants from disease and the potential to prevent the pathogen reproducing and spreading. Most of the research on this fungicide has concentrated on
horticultural crop species, whilst few studies have examined the phosphite treatment of native Australian plants.

In conjunction with Murdoch University the present study was instigated by Alcoa World Alumina, Worsley Alumina, Iluka Resources, Tiwest Joint Venture, Worsley Alumina and Sons of Gwalia, together with the support of the Minerals and Energy Research Institute of Western Australia and the Australian Research Council to: ‘Examine the potential of the fungicide phosphite to control Phytophthora cinnamomi in rehabilitated minesites and adjacent native plant communities.’

Specifically the aims of the project were to:

1. assess the beneficial uses of phosphite to:
   • prevent deaths of a wide range of plant species by increasing their resistance to P. cinnamomi, and
   • minimise the spread of P. cinnamomi by preventing sporulation in infected plants.

2. assess the potential detrimental impacts of using phosphite in native plant communities by studying its:
   • effects on plant health and reproduction, and
   • effects on mycorrhizal fungi

3. develop practicable and economic field methods to treat native vegetation with phosphite to maximise the beneficial aspects of its uses and minimise any detrimental impacts.

The expected outcomes of this study were the development of a set of procedures for the application of phosphite in a range of native plant communities, including information on rate of application, frequency of application, season of application and a description of the expected benefits.
In native plant species there is a paucity of information on the persistence of phosphite in controlling *P. cinnamomi* over time after application. Shearer and Fairman (1997b) found that injection of *Banksia grandis* and *Eucalyptus marginata* with 50, 100 and 200 g phosphite/L controlled lesion extension of *Phytophthora cinnamomi* in wound inoculated plants for at least four years after treatment. Similarly, injection of *Banksia attenuata* with 100 g phosphite/L protected trees, growing in a disease front, for up to four years. Shearer and Fairman (1997a) found that foliar application of 5 g phosphite/L increased the time to 50% mortality of three species of *Banksia* growing along a *P. cinnamomi* disease front by an average of 2-6 years depending on the species treated. In another study, foliar application of 6 g phosphite/L prevented deaths of *Xanthorrhoea australis* for at least two years in *P. cinnamomi* infected vegetation (Aberton et al. 1999). However, it is estimated that there are over 9000 plant species native to the southwest of Western Australia and there is almost no information on the efficacy and longevity of phosphite in these species.

The aim of the following trials was to increase the number of plant species and for which we have information on the response to phosphite treatment. There were two field trials. The first (Chapter 2.1) examined the longevity and efficacy of phosphite in a range of plant species from two plant communities; the jarrah forest and the northern sandplain. Whilst glasshouse trials (Chapter 2.2) were run in conjunction with the field trials to increase the number of plant species studied. The second field trial (Chapter 2.3) examined the long-term efficacy of phosphite to prevent deaths in a range of plant species that have been both wound inoculated with *P. cinnamomi* and had the soil surrounding the plants inoculated with the pathogen.
2.1 The long term ability of phosphite to control *Phytophthora cinnamomi* in two native plant communities of Western Australia

(to be submitted to Australian Journal of Botany for publication)

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Abstract

This study examined the ability of foliar applications of the fungicide phosphite to contain the colonisation of *Phytophthora cinnamomi* in a range of plant species from two native plant communities in south-western Australia. Plants species within and between plant communities varied considerably in their ability to take up and retain phosphite in inoculated stems and in the levels of phosphite required *in planta* to contain *P. cinnamomi*. As phosphite levels applied increased from 5 to 20 g/L, stem tissue levels increased, as did the ability of a plant species to contain *P. cinnamomi*. However, above 5 g phosphite/L, phytotoxicity symptoms tended to be high in most species with some being killed. So despite, 10 and 20 g/L phosphite being more effective and persistent in the control of *P. cinnamomi*, they are not recommended rates for application to plant communities. The maximum levels of phosphite measured in stems varied between species. For example, for plants sprayed with 5 g phosphite/L, the inoculated stem phosphite levels varied between 26 ug/g dry wt in *Leucopogon verticillatus* and 299 ug/g dry wt for *Hibbertia furfuracea*. Whilst after an application of 10 g phosphite/L the average phosphite levels varied between 41 ug/g dry wt in *L. verticillatus* and 668 ug/g dry wt in *H. furfuracea*. Responses to phosphite also varied between season of application, with some species being more responsive after a spring and others more responsive after an autumn application of phosphite. In *Banksia grandis*, phosphite was not detectable in the inoculated stems 24 months after application but *P. cinnamomi* growth was still contained. In contrast, 12 months after phosphite application to *Leucopogon verticillatus* phosphite levels were still detected in inoculated stems, but *P. cinnamomi* growth was no longer contained. This study indicates that foliar applications of phosphite have considerable potential in native plant communities to reduce the impact of *P. cinnamomi* in the short-term. However, in order to maintain adequate control phosphite should be sprayed every 6-12 months.

Introduction

*Phytophthora cinnamomi* Rands is a major pathogen in native plant communities in Western Australia (Dell *et al.* 1989; Wills 1993). It affects approximately 14% of the northern jarrah (*Eucalyptus marginata* Sm.) forest in Western Australia (Davison *et al.* 1989) and over 2000 of the 9000 plant species in the *Banksia* woodlands and heathlands of the south-west of Western Australia may be susceptible (Wills 1993). Mining operations and timber harvesting are major activities in areas where *P. cinnamomi* is prevalent. The presence of *P. cinnamomi* in and around mining operations increases the financial cost for most mines. Hence, the development of a method to contain or eradicate the pathogen would be a great financial and environmental advantage.
Fungicides have rarely been used to control diseases in native plant communities due to high cost and phytotoxic responses, other control measures have been developed in preference (Colquhoun et al. 1994). Research has shown that neutralised phosphorus acid (phosphite) has value in conserving rare and endangered plant species in the south-west of Western Australia (Shearer et al. 1991).

There is confusion in the literature as to the correct use of the name phosphite or phosphonate. We have chosen to use phosphite to describe the chemical containing a P-H bond, as it describes the compound commercially available in Australia, a phosphite salt (mono-di-potassium phosphite).

Phosphite controls many plant diseases caused by Phytophthora, even at concentrations in planta which only partially inhibit pathogen growth in vitro. Phosphite is systemically translocated in both the xylem and phloem (Guest et al. 1991; Guest et al. 1995). This property along with its good water solubility, enables it to be applied either as a foliar spray (Guest et al. 1991), as a soil drench (Smillie et al. 1989) or as a trunk injection (Pegg 1990; Guest et al. 1995) against a wide range of diseases caused by Phytophthora spp.

Most research involving phosphite has concentrated on horticultural crop species. Few have examined the use of phosphite on native Australian plants. The purpose of this study was to determine the long-term efficacy of foliar application of phosphite on a range of Australian plant species in the jarrah forest and northern sandplain of Western Australia.

**Methods**

**Phosphite treatment for control of P. cinnamomi in stems of jarrah forest species**

*Trial design and plant species.* The experiment was a complete randomised design established in the jarrah (Eucalyptus marginata) forest near Dwellingup, Western Australia (Alcoa World Alumina Australia Huntly mine 32°42’41’S, 116°03’29’E). The treatments were; season of phosphite application, phosphite spray concentration, and plant species. Plants from several families with a range of known susceptibilities to *P. cinnamomi* were used. These were *Banksia grandis* Willd. (Proteaceae), *Daviesia physodes* Don (Papilionaceae), *Lasiopetalum floribundum* Benth. (Sterculiaceae), *Leucopogon verticillatus* R.Br. (Epacridaceae) and *Trymalium ledifolium* Fenzl (Rhamnaceae). Plants ranged from 30 cm to 1.5m in height and there was a range of small, medium and large plants included in each treatment combination for each species.

*Phosphite application.* Backpack spray units (Volpi 15L) were used to treat plants in spring (November 1996) or autumn (May 1997) with phosphite (Fosject 200 or Fol-R-Fos 400, active ingredient mono-di potassium phosphite, manufactured by Unitec Group Pty Ltd) and 0.25% of the adjuvant, Synertrol Oil (Organic Crop Protectants). Plant foliage was sprayed to run-off with 0 (control), 5, 10 or 20 g phosphite/L in spring. As severe phytotoxicity symptoms were observed after application of 20 g phosphite/L in spring this treatment was omitted in the autumn spray.

*Inoculum production.* Miracloth (Calbiochem® - Novabiochem Corporation La Jolla, CA) discs
(6 mm diameter) colonised with *P. cinnamomi* isolate MP94-48 were used as inoculum. The isolate was obtained from a diseased *E. marginata* growing in a rehabilitated bauxite mine (Alcoa World Alumina) in the south-west of Western Australia and was passaged through a plant of this species prior to each inoculation event to ensure it was still able to colonise plant tissue. The Miracloth discs were washed thoroughly with deionised water, autoclaved three times and placed on 10% V8 agar (Byrt et al. 1979). A square of colonised agar was cut from the growing edge of a *P. cinnamomi* culture and placed on the centre of the plate. After 10 days the Miracloth discs were colonised by *P. cinnamomi* and were used to inoculate plants.

**Plant inoculation.** The main plant stem or a lateral branch was underbark inoculated by the method of O’Gara et al. (1996). Briefly, the stem was cut through the periderm to the phloem in an upward movement using a scalpel blade and a colonised Miracloth disc was inserted into the phloem wound. The wound was sealed with Parafilm (American National Can™ Chicago IL) and silver insulation tape to prevent desiccation. One stem, at least 20cm above the ground and wherever possible with a further 20 cm of stem above the inoculation point, was inoculated on each plant.

Seven (spring spray) or eleven (autumn spray) plants of each species by phosphite concentration combination were inoculated at five time intervals in relation to the time phosphite was applied. Plants sprayed in spring were inoculated 10 days prior to phosphite application or 8 days, 5 months, 12 months or 24 months after phosphite was applied. Plants sprayed in autumn were inoculated 10 days prior to phosphite application or 2 weeks, 6 months, 12 months or 24 months after phosphite was applied. Inoculation times were chosen to coincide with the environmental conditions in which *P. cinnamomi* is normally active.

**Plant harvest.** Plants were harvested 8 days to 5 weeks after they were inoculated depending on species, season and visual lesion extension. At harvest, the inoculated stems were removed and, depending on the plant species, 18 to 28 cm of the stem above the inoculation point was cut sequentially into 1 cm segments. Each segment was cut longitudinally in half and placed, cut surface down, on a *Phytophthora* selective medium ((Shearer et al. 1995), modified by the addition of 10 mg/L Rifampicin (Rifadin, Hoescht Marion Ruessel, Italy)) to determine the growth of *P. cinnamomi*. The growth rate of *P. cinnamomi* was calculated by dividing the maximum distance that *P. cinnamomi* grew from the inoculation point by the number of days that the plants were inoculated.

**Phosphite analyses.** *Banksia grandis*, *Daviesia physodes* and *Leucopogon verticillatus* stems were analysed for phosphite using high performance ion chromatography (HPIC) (Roos et al. 1999). At each harvest, 20 cm of the stem below the inoculation point was washed with a phosphate free detergent, rinsed with distilled water, dried in a 30°C oven for one month and ground for HPIC analysis (Roos et al. 1999). When phosphite was applied in spring, plants were harvested 15 days or approximately 6, 13 or 25 months after application and analysed, whilst plants treated with phosphite in autumn were harvested 11
Phosphite treatment for control of *P. cinnamomi* in stems of plant species from the northern sandplain

**Trial design and plant species used.** The experiment was a complete randomised design established in the northern sandplain near Eneabba, Western Australia (Iluka Resources Ltd mine, 29°49'10"S, 115°16'18"E). The treatments were: season of phosphite application, phosphite spray concentration, and plant species. Plants belonging to several families known to be mainly susceptible to *P. cinnamomi* were used. These were *Hakea flabellifolia* Meisn. and *Lambertia multiflora* Lindl. (Proteaceae), *Hibbertia furfuracea* (DC.)Benth (Dilleniaceae) and *Jacksonia floribunda* Endl. (Papilionaceae). Four additional species, which were present in low numbers in the area, were included for two inoculation times; *Astroloma xerophyllum* (DC.)Sond. (Epacridaceae), *Banksia* sp. (Proteaceae) and *Eremaea beaufortioides* Benth. and *Verticordia grandis* J.L.Drumm. (Myrtaceae). Due to the nature of the heathland area there was a wide variation in plant age. However, size within a species was kept relatively constant.

**Phosphite application.** Backpack spray units were used to treat plants in spring (September 1997) or autumn (May 1998) with phosphite (Fol-R-Fos 400), and 0.25% of the adjuvant, Synertrol Oil. Plant foliage was sprayed to run-off with 0 (control), 5 or 10 g phosphite/L.

**Plant inoculation and harvest.** Miracloth discs colonised with *P. cinnamomi* isolate MP97-20 was used as inoculum. The isolate was obtained from a diseased *Lambertia multiflora* growing on the minesite and prior to each inoculation time it was passaged through an *Eucalyptus marginata* plant to ensure that it was still able to colonise plant tissue. Plants were underbark inoculated using the method described above. Plants were inoculated on the southern side of the stem to limit the amount of direct sunlight on the inoculation point, since temperatures above 30°C are normal in the trial area in spring and summer.

Eleven plants of each species by phosphite concentration combination were inoculated at each time period after phosphite was applied. Plants treated with phosphite in spring were inoculated 7 days prior to phosphite application or 12 days, 9 months, 12 months or 24 months after phosphite was applied. Plants treated with phosphite in autumn were inoculated 6 days prior to phosphite application or 2 weeks, 4 months, 12 months or 18 months after phosphite was applied. The four additional plant species were inoculated 8 or 12 months and 4 or 12 months after the spring and autumn spray applications, respectively. In each case, plants were harvested 3 to 6 weeks after inoculation depending on species, season and visual lesion extension. At harvest, plants were treated as described above.

**Phosphite analyses.** Stems of *Hibbertia furfuracea* and *Lambertia multiflora* plants that were sprayed in spring were analysed for phosphite after the first and second or the second inoculation, respectively. For *H. furfuracea*, 20 cm of a stem of similar diameter to the inoculated stem and for *L. multiflora* 20 cm

and 40 days or approximately 7, 13 or 24 months after application and analysed.
below the inoculation point was analysed. These plants were harvested 14 or 34 days after phosphite was applied.

Phytotoxicity assessment
For both trials (sandplain and jarrah forest) plants were ranked according to the extent of foliar damage at 2-7 weeks after spray application. A scale with 4 broad categories was used as follows: 0 = no damage, + = <25% canopy with necrotic symptoms, ++ = 26 – 75% canopy with necrotic symptoms, and +++ = 76 – 100% of canopy with necrotic symptoms. The ranking system integrated symptom development for the whole canopy. For example, ++ does not distinguish between plants that had 50% of leaves with 100% necrosis and plants in which all leaves had 50% necrosis. In addition, the condition of the canopy was reassessed from 1.5 to 3 years after spray application. The number of dead plants, degree of stem death on living plants, re-sprouting and apical growth were recorded.

Temperature and rainfall
Ambient temperature and rainfall data was obtained from station number 9538, Dwellingup Forestry (jarrah forest trial) and station number 8225, Eneabba Post Office (sandplain trial).

Statistical analysis
The growth rate data for each season of spray application (spring or autumn) for each plant species were analysed using analysis of covariance with the independent variables spray concentration and time after phosphite was applied. Growth rate data were transformed (square root, log (x+0.01), inverse log) when necessary to make residuals homoscedastic and approximately normal. The Least Squares Means test was used ($\alpha=0.05$) to determine the difference in growth rate of $P. cinnamomi$ at the phosphite spray concentrations at each inoculation time. The covariates used were plant size and stem diameter at the point of inoculation.

Results
To simplify the discussion of the data the responses of Banksia grandis in the jarrah forest and Lambertia multiflora in the sandplain to phosphite and $P. cinnamomi$ are referred to in detail. The other species are then compared to these. The $B. grandis$ was selected because it is the species in Western Australia on which most of the previous studies have been made on the efficacy of phosphite to control $P. cinnamomi$. The $L. multiflora$ was chosen because it is a member of the Proteaceae, the same family as $B. grandis$, and it is a dominant member of the northern sandplain community and it is susceptible to $P. cinnamomi$. Unless stated otherwise, the level of significance for differences was $p<0.05$ and are a comparison between plants treated with phosphite and control plants (0 g phosphite/L).
Phosphite treatment for control of *P. cinnamomi* in stems of jarrah forest species

*Spring phosphite application.* When phosphite was applied to *Banksia grandis* 10 days after they were inoculated there was no significant reduction in the growth rate of *P. cinnamomi* (Figure 2.1a). When they were inoculated after phosphite was applied there was a significant reduction in the growth rate of *P. cinnamomi* for at least 12 months. However, 24 months after phosphite was applied, the growth rate of *P. cinnamomi* was only reduced significantly in *B. grandis* treated with 20 g phosphite/L. While there was a reduction in the growth rate of *P. cinnamomi* in *B. grandis* treated with 5 or 10 g phosphite/L, this reduction was not significant.

As with *Banksia grandis*, when phosphite was applied to *Daviesia physodes*, *Leucopogon verticillatus*, *Lasiopetalum floribundum* and *Trymalium ledifolium* 10 days prior to inoculation of the plants there was no significant reduction in the growth rate of *P. cinnamomi* in any of the species (Figure 2.1a). There was a reduction in *P. cinnamomi* growth rate in all of these species when they were inoculated 8 days after phosphite was applied. However, this reduction was not significant in *L. verticillatus* sprayed with 20 g phosphite/L, *D. physodes* sprayed with 5 g phosphite/L and in *L. floribundum* and sprayed with 5 or 10 g phosphite/L. Five months after phosphite was applied the growth rate of *P. cinnamomi* was significantly reduced in *D. physodes*, *L. verticillatus*, *L. floribundum* and *T. ledifolium* which had been sprayed with 20 g phosphite/L. In contrast, there was no significant reduction in the growth rate of *P. cinnamomi* in these species sprayed with 5 or 10 g phosphite/L except in *T. ledifolium* sprayed with 5 g phosphite/L. There was no significant difference in the growth rate of *P. cinnamomi* in *D. physodes*, *L. verticillatus*, *L. floribundum* and *T. ledifolium* inoculated 12 months after phosphite was applied. However, as the growth rate in the control plants was very slow it was difficult to draw any conclusions from results for this inoculation time. Finally, 24 months after phosphite was applied there was no significant difference in the growth rate of *P. cinnamomi* in *D. physodes* or *L. verticillatus*. No comment can be made about the efficacy of phosphite in *L. floribundum* as there was no growth in the control plants. Whilst for *T. ledifolium* all of the remaining plants that had been sprayed with 5-20 g phosphite/L had died.
Figure 2.1a. The effect of foliar application of phosphite (0, 5, 10 or 20 g/L) applied in spring (n=7) on the average growth rate of Phytophthora cinnamomi in the stems of plant species from the Eucalyptus marginata forest of Western Australia. Points with different letters indicate a significant (p<0.05) difference in the growth rate of P. cinnamomi within each plant species and time of inoculation.
Figure 2.1b. The effect of foliar application of phosphite (0, 5, 10 or 20 g/L) applied in autumn (n=11) on the average growth rate of *Phytophthora cinnamomi* in the stems of plant species from the *Eucalyptus marginata* forest of Western Australia. Points with different letters indicate a significant (p<0.05) difference in the growth rate of *P. cinnamomi* within each plant species and time of inoculation.

In *Banksia grandis* stems harvested 15 days after phosphite was applied (inoculated 8 days after phosphite application) the phosphite levels increased as the phosphite concentration applied increased, and ranged between 36-100, 36-247 and 96-346 µg g⁻¹ dry weight for plants treated with 5, 10 or 20 g phosphite/L, respectively. Five months after phosphite was applied, phosphite was barely detectable (Figure 2.2a), yet there was still a significant reduction in the growth rate of *P. cinnamomi* in these plants (Figure 2.1a). At the higher concentrations of spray very low levels of phosphite were detected in *B. grandis* after 12 and 24 months.
Figure 2.2. The average phosphite concentration (ug g⁻¹ dry wt ± SE) in the stems of plant species from the *Eucalyptus marginata* forest which were treated with phosphite in (a) spring or (b) autumn. 0 g phosphite/L (n=2), 5, 10 and 20 g phosphite/L (n=6).

The range of phosphite concentrations in the stems of *Daviesia physodes* decreased markedly between the 8 day and 5 month inoculation times (Figure 2.2a). This decrease in detectable phosphite corresponded to the time when the growth rate of *P. cinnamomi* in plants treated with phosphite became comparable with the control plants (Figure 2.1a). Phosphite was more persistent in the stems of...
Leucopogon verticillatus with detectable levels present up to 12 months after phosphite was applied. However, *P. cinnamomi* growth was not contained in *L. verticillatus* after 5 months. 

**Autumn phosphite application.** There was a significant reduction in the growth rate of *P. cinnamomi* in Banksia grandis when they were inoculated prior to treatment with 10 g phosphite/L, which contrasted to the spring application where there was no control. When they were inoculated after phosphite was applied there was a significant reduction in growth rate of *P. cinnamomi* at all phosphite concentrations up to 6 months after treatment. Twelve and 24 months after phosphite was applied, the growth rate of *P. cinnamomi* was significantly reduced in *B. grandis* treated with 10 g phosphite/L but not in those treated with only 5 g phosphite/L.

When Daviesia physodes, Leucopogon verticillatus, Lasiopetalum floribundum and Trymalium ledifolium were inoculated with *P. cinnamomi* 10 days before phosphite was applied, no concentration of phosphite reduced the growth of *P. cinnamomi*. These are similar to the observations made for these species treated in spring. When these species were inoculated 2 weeks to 12 months after phosphite was applied there was a significant reduction in the growth rate of *P. cinnamomi* in all species except *L. floribundum*. However, the growth rate of *P. cinnamomi* in the *L. floribundum* control plants was very slow for all inoculation times thus no comment can be made on the efficacy of phosphite in this species. Twenty-four months after phosphite was applied there was no significant reduction in *P. cinnamomi* growth rate in *D. physodes, L. verticillatus, L. floribundum* and *T. ledifolium*. However, the growth rate in the controls was slow so it was difficult to draw conclusions from these results. Twenty-four months after phosphite was applied all remaining *T. ledifolium* which had been sprayed with 5 or 10 g phosphite/L were dead, thus this species was not included in the final inoculation time.

There was a rapid decrease in the phosphite levels in *B. grandis* stems between 11 and 40 days after it was applied (inoculated 10 days prior and 2 weeks after phosphite was applied) (Figure 2.2b). However, the growth rate of *P. cinnamomi* was significantly reduced in *B. grandis* for at least 24 months after phosphite was applied, this was a similar observation to the plants sprayed in spring. Detectable levels of phosphite persisted in the stems of *D. physodes* and *L. verticillatus* for longer than they did in *B. grandis* (Figure 2.2b).

**Temperature and rainfall data.** The average minimum and maximum temperatures over the times the plants were inoculated ranged from 6.8-15.4°C to 16.6-33.1°C, respectively (Table 2.1). The lowest minimum (0°C) and lowest average maximum (16.6°C) occurred during the autumn inoculation 24 months after phosphite was applied. These low temperatures correspond to the slow growth rate of *P. cinnamomi* in the control plants of all the plant species (2.1b). The total rainfall for the 6 weeks prior to and during each inoculation ranged from 52-510 mm (Table 2.1).
Table 2.1. Jarrah forest trial of phosphite application. Environmental conditions before inoculation and between inoculation and harvest.

<table>
<thead>
<tr>
<th>Season of spray application</th>
<th>Time^A prior to inoculation (mm)</th>
<th>Rainfall^B (mm)</th>
<th>Conditions between inoculation and harvest (1-5 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lowest minimum °C</td>
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<tr>
<td>Spring</td>
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</tr>
<tr>
<td></td>
<td>24 months after</td>
<td>203</td>
<td>5.0</td>
</tr>
<tr>
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<td>10 days prior</td>
<td>46</td>
<td>3.0</td>
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<td>2 weeks after</td>
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<td>172</td>
<td>0.0</td>
</tr>
</tbody>
</table>

^A Time of inoculation relative to phosphite application.

^B Rainfall in the 6 week period prior to plant inoculation.

Phosphite treatment for control of *P. cinnamomi* in stems of plants from the northern sandplain

*Spring phosphite application.* There was a significant reduction in the growth rate of *P. cinnamomi* in *Lambertia multiflora* when they were inoculated prior to and 12 days after phosphite was applied (Figure 2.3a). The growth rate of *P. cinnamomi* was significantly reduced for at least 12 months when *L. multiflora* were sprayed with 10 g phosphite/L, though the data at 9 months were not significant. Twenty-four months after phosphite was applied there was no significant reduction in the growth rate of *P. cinnamomi* at either phosphite concentration.

The results for *Hibbertia furfuracea* were similar to *Lambertia multiflora* in that an effect could be detected if plants were inoculated before they were sprayed, and up to 12 months later when sprayed after they were inoculated (Figure 2.3a). Application of phosphite had no effect on the growth rate of *P. cinnamomi* in *Hakea flabellifolia* at any inoculation time. In *Jacksonia floribunda* there was a significant reduction in the growth rate of *P. cinnamomi* 12 days and 12 months after phosphite was applied. Phosphite did not effect the growth rate of *P. cinnamomi* in *H. flabellifolia* stems.
Figure 2.3. The effect of foliar application of phosphite (0, 5 or 10 g/L) applied in (a) spring or (b) autumn on the average growth rate of *Phytophthora cinnamomi* in the stems of plant species from the northern sandplain of Western Australia. Points with different letters indicate a significant (p<0.05) difference in the growth rate of *P. cinnamomi* within each plant species and time of inoculation (n=11).
The average phosphite concentration in the stems of *Lambertia multiflora* which were sprayed with 5 and 10 g phosphite/L and harvested 34 days later was 17 (SE ± 6) or 68 (SE ± 32) µg g⁻¹ dry stem weight, respectively. The average phosphite concentration in the stems of *Hibbertia furfuracea* which were sprayed with 5 or 10 g phosphite/L and harvested 14 days after phosphite was applied was 299 (SE ± 57) or 668 (SE ± 109) µg g⁻¹ dry stem weight, respectively. When plants were harvested 34 days after phosphite was applied the levels of phosphite had decreased approximately 3-fold to 96 (SE ± 18) or 219 (SE ± 43) µg g⁻¹ dry stem weight in plants treated with 5 or 10 g phosphite/L, respectively.

**Autumn phosphite application.** The growth rate of *P. cinnamomi* was significantly reduced in *Lambertia multiflora* sprayed with 5 g phosphite/L, 6 days prior to inoculation (Figure 2.3b). Application of phosphite reduced the growth rate of *P. cinnamomi* for up to 4 months in *L. multiflora* sprayed prior to inoculation. No comment can be made about the ability of phosphite to decrease the rate of *P. cinnamomi* growth when plants were inoculated prior to phosphite application as the growth rate in the control plants was very slow (Figure 2.3b). This is also the case for all inoculations of *Jacksonia floribunda* (data not shown) and for the 18-month inoculation of *Hakea flabellifolia*. In *Hibbertia furfuracea* there was a significant decrease in the growth rate of *P. cinnamomi* in plants sprayed 2 weeks, 4 months (10 g phosphite/L only) and 18 months (10 g phosphite/L only) prior to inoculation. Finally, in *H. flabellifolia* there was only a significant reduction of growth rate of *P. cinnamomi* in plants treated with 10 g phosphite/L 2 weeks and 4 months prior to inoculation.

Phosphite did not affect the growth rate of *P. cinnamomi* in *Eremaea beaufortioides* or *Verticordia grandis* when it was applied in spring or autumn (Figure 2.4a and b). In *Banksia* sp. there was a significant reduction in the growth rate of *P. cinnamomi* in plants treated with 10 g phosphite/L, 8 months (spring and autumn spray) and 5 g phosphite/L, 12 months (spring spray only) after phosphite was applied. All *Astroloma xerophyllum* treated with 10 g phosphite/L in spring died.
Figure 2.4. The effect of foliar application of phosphite applied in (a) spring or (b) autumn on the average growth rate of *Phytophthora cinnamomi* in the stems of plant species from the northern sandplain of Western Australia. Points with different letters indicate a significant (p<0.05) difference in the growth rate of *P. cinnamomi* within each plant species and time of inoculation (n=11).

**Temperature and rainfall data.** The average minimum and maximum temperatures during the inoculation times ranged from 8.2-14.4°C to 18.2-27.8°C, respectively (Table 2.2). The lowest minimum (0.5°C) and the lowest average maximum (18.2°C) temperatures were recorded during the 9-month inoculation of the spring sprayed plants. These low temperatures correspond to a slow growth rate of *P. cinnamomi* in the control plants in all plant species (Figure 2.2a). The total rainfall for the 6 weeks prior to and during each inoculation ranged from 76.2–345 mm (Table 2.2). The lowest rainfall (3.4 mm) in the 6 weeks prior to plants being inoculated occurred before the autumn sprayed plants were inoculated 6 days prior to phosphite being applied. This low rainfall also corresponds to a slow growth rate of *P. cinnamomi* in the control plants in all plant species (Figure 2.2b).
Table 2.2. Northern Sandplain trial of phosphite application. Environmental conditions before inoculation and between inoculation and harvest.

<table>
<thead>
<tr>
<th>Season of spray application</th>
<th>Time(^{A})</th>
<th>Rainfall(^{B})</th>
<th>Conditions between inoculation and harvest (1-5 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rainfall (\text{mm})</td>
<td>Lowest (\text{°C})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>prior to inoculation</td>
<td>minimum</td>
</tr>
<tr>
<td>Spring</td>
<td>7 days prior</td>
<td>113</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>12 days after</td>
<td>71</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>8 months after</td>
<td>43</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>9 months after</td>
<td>170</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>12 months after</td>
<td>80</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>24 months after</td>
<td>101</td>
<td>5.0</td>
</tr>
<tr>
<td>Autumn</td>
<td>6 days prior</td>
<td>3</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>2 weeks after</td>
<td>43</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>4 months after</td>
<td>85</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>12 months after</td>
<td>123</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>28 months after</td>
<td>115</td>
<td>5.0</td>
</tr>
</tbody>
</table>

\(^{A}\)Time of inoculation relative to phosphite application.

\(^{B}\)Rainfall in the 6 week period prior to plant inoculation.

Phytotoxicity assessment

In general, application of phosphite in spring caused more short and long-term phytotoxicity symptoms (Table 2.3). There was a range of phytotoxicity symptoms seen 1-month after phosphite was applied. These included necrosis of leaf tips and margins, patches on leaves, base of leaves and/or the leaf pedicel, stems and leaf drop. When plants in the jarrah forest and the sandplain were sprayed with phosphite in spring it caused necrosis on the flowers of *Astroloma xerophyllum*, *Hibbertia furfuracea* and *Lasiopetalum floribundum* and burnt the fruit peduncle which caused fruit drop of *Eremaea beaufortioides* and *Trymalium ledifolium*.

Application of 5 g phosphite/L caused, in general, less than 25% damage to the plant canopy (Table 2.3). Plant species which were most severely affected (76-100% of the canopy damaged) by this concentration of phosphite were; *Trymalium ledifolium* and *Astroloma xerophyllum* when sprayed in spring. Application of 10 g phosphite/L caused up to 76% damage to the plant canopy depending on the plant species treated and the season of application. Species most severely affected were *T. ledifolium* and *A. xerophyllum* sprayed in spring or autumn. *Hibbertia furfuracea* was badly affected when sprayed in spring and *Verticordia grandis* when sprayed in autumn. Treatment with 20 g phosphite/L were only used in the jarrah forest. This concentration resulted in necrosis on all species with the most severely affected species being *Daviesia physodes* sprayed in spring or autumn and *Leucopogon verticillatus* sprayed in autumn (Table 2.3).
When plants were assessed 1½ years or more after phosphite was applied, phytotoxic effects of the spray were seen as decreased apical growth, stem or plant death and sprouting from the base or mid-canopy (Table 2.3). In the species that were affected, the level of phytotoxicity increased as the phosphite concentration increased. Almost all long-term symptoms were only observed on plants sprayed with a phosphite concentration greater than 5 g/L. *Astroloma xerophyllum* and *Trymalium ledifolium* were the worst affected species with phosphite causing a large number of plant deaths. For *A. xerophyllum* phosphite caused more damage when it was applied in spring than autumn. Phosphite caused little to no damage to *Banksia grandis*, in the jarrah forest, and *Banksia* sp., *Jacksonia floribunda*, *Lambertia multiflora* and *Verticordia grandis*, in the sandplain.

**Discussion**

Foliar application of phosphite was shown to significantly reduce the colonization of plant stems by *P. cinnamomi* if they are infected for up to 5 to 24 months after phosphite was applied, depending on the plant species sprayed and the rate of application. However, the higher levels of phosphite (10 or 20 g/L) were phytotoxic to most species and lethal for some species. When plants were inoculated with *P. cinnamomi* prior to phosphite application the results were more variable. For example, in only one of the jarrah forest and two of the northern sandplain species tested, did phosphite treatment result in the containment of *P. cinnamomi* growth. Thus, phosphite application in areas where plants are already infected, it is unlikely to give satisfactory control in many plant species. Application of phosphite to diseased plant communities is probably only justified in the short-term to conserve rare and endangered plant species. Our results disagree with Shearer and Fairman (1997a) who showed that foliar application of 5 g phosphite/L increased the time to 50% mortality of three species of *Banksia* growing in a *Phytophthora* infested area by 2-6 years, depending on the species treated. However, plants were repeat-sprayed after 2.5 years. They also recorded deaths whilst we examined colonisation. In a separate experiment Shearer and Fairman (1997b) examined the longevity of the efficacy of phosphite in controlling colonisation of *P. cinnamomi* in trunk injected *Banksia grandis*. They found that colonisation was reduced for at least four years, which a similar result to their spray application.

There was a large variation between the plant species in the rate of colonisation of stems by *P. cinnamomi* despite the fact that they had all been selected because they were susceptible to *P. cinnamomi* in the field. Susceptibility in the field is based on death as a result of root colonisation and girdling of the conducting tissues. For example, *Trymalium ledifolium* is used as a “*Phytophthora*” indicator species in the jarrah forest yet in our trials there was little growth of the pathogen in inoculated stems. It is possible that the stems of some of species such as *T. ledifolium* and *Lasiorpetalum floribundum* are more tolerant to *P. cinnamomi* than their roots. Therefore, in future preliminary studies should be conducted to confirm that stems are as susceptible as roots before relying on stem inoculation.

The persistence in the ability of phosphite to contain *P. cinnamomi* varied between plant species, season of phosphite application and plant community. For example, in *Banksia grandis* by 12 months after the
Table 2.3. Effect of phosphite rate and time of application on visible phytotoxicity at 2 to 7 weeks and plant growth after >1.5 years after application.

Categories used for rating phytotoxicity; 0=no damage and +=<25%, ++=26-75% and +++=76-100% canopy with necrotic symptoms.

<table>
<thead>
<tr>
<th>Species</th>
<th>Season</th>
<th>Phytotoxicity ratinga</th>
<th>Effect on growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phosphite concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(g/L)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0  5  10  20</td>
<td></td>
</tr>
<tr>
<td><strong>Jarrah forest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Banksia grandis</em></td>
<td>Spring</td>
<td>0  +  +  ++</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>+  +  +  +</td>
<td>None</td>
</tr>
<tr>
<td><em>Daviesia physodes</em></td>
<td>Spring</td>
<td>0  +  ++  +++</td>
<td>10 and 20 g/L increased plants with &gt;50% stem death by ca. 80%; 20 g/L increased plant death by 40%</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>+  +  ++  +++</td>
<td>5, 10 and 20 g/L increased plants with &gt;50% stem death by ca. 25, 75 and 80%, respectively; 10 g/L and 20 g/L increased plant death by 15 and 35%, respectively</td>
</tr>
<tr>
<td><em>Lasiopetalum floribundum</em></td>
<td>Spring</td>
<td>0 0 0 +</td>
<td>Phosphite increased resprouting from rootstock; 10 and 20 g/L increased plants with &gt;50% stem death by ca. 60 and 100%, respectively</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>+  +  +  ++</td>
<td>phosphite increased resprouting from rootstock; 10 and 20 g/L increased plants with &gt;50% stem death by ca. 50 and 90%, respectively; 20 g/L increased plant death by 15%</td>
</tr>
<tr>
<td><em>Leucopogon verticillatus</em></td>
<td>Spring</td>
<td>0  +  ++  ++</td>
<td>10 and 20 g/L increased plants with &gt;50% stem death by 50 and 80%, respectively</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>+  ++  ++  +++</td>
<td>20 g/L increased plants with &gt;50% stem death by ca. 10%</td>
</tr>
<tr>
<td><em>Trymalium ledifolium</em></td>
<td>Spring</td>
<td>0  +++  +++  +++</td>
<td>5 – 20 g/L increased plant death by 50%</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>+  +  +++  +++</td>
<td>10 and 20 g/L increased plant death by 35 and 100%, respectively</td>
</tr>
<tr>
<td>Species</td>
<td>Season</td>
<td>Spring</td>
<td>Autumn</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td><em>Banksia sp</em></td>
<td></td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><em>Eremaea beaufortioides</em></td>
<td>Spring</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><em>Hibbertia furfuracea</em></td>
<td>Spring</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><em>Hakea flabellifolia</em></td>
<td>Spring</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Jacksonia floribundum</em></td>
<td>Spring</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Astroloma xerophyllum</em></td>
<td>Spring</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td><em>Lambertia multiflora</em></td>
<td>Spring</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Verticordia grandis</em></td>
<td>Spring</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0</td>
<td>++</td>
</tr>
</tbody>
</table>
autumn application of 5 g phosphite/L there was no longer any control of *P. cinnamomi* growth but plants sprayed in spring with the same concentration was still able to restrict growth of the pathogen. In contrast, in *Daviesia physodes* the autumn application of 5 g phosphite/L and above reduced *P. cinnamomi* colonisation for longer than 12 months, but only for 5 months after the spring application. Similar differences were observed for other species. It is likely, that environmental and host factors such as temperature, plant water status and growth stage will all influence the efficacy of phosphite, the ability of the pathogen to invade the host and the plants’ ability to respond to the pathogen. To date, there is a paucity of detailed research on host, pathogen and environment interactions in native plant species, let alone with these interactions after the addition of phosphite. However, our results do indicate that there was considerable variation in the extent of *P. cinnamomi* colonisation when plants were inoculated in different seasons. For example, in the jarrah forest, when the average minimum and maximum temperatures were 7.2 and 16.6°C (24 months after the autumn spray application), respectively, the rate of *P. cinnamomi* colonisation was very slow in *B. grandis* and the other species tested. Small increases in average temperature increased the rate of colonisation. For example, in the jarrah forest, when the maximum and minimum temperatures were 9.5 and 18.0°C (10 days prior to the autumn spray application), respectively, *P. cinnamomi* colonisation was moderate in *B. grandis*.

Low stem water status is known to reduce the colonisation by *P. cinnamomi* in plants (Tippett *et al.* 1987; Bunny *et al.* 1995). In the present study, rainfall immediately prior to inoculation varied between 35mm to 203 mm, and 71 to 170 mm in the jarrah forest and northern sandplain, respectively. Subsequent colonisation by *P. cinnamomi* in the non-phosphite treated plants was not always greatest in those periods that received the most rainfall. Therefore, plant water status did not appear to be influencing *P. cinnamomi* colonisation, whilst temperature did.

It is likely that the uptake of phosphite into a particular plant species will vary according to temperature, growth stage of the host, plant water status and morphological factors of the host such as location of stomata, leaf hairiness, cuticle thickness and sensitivity to phosphite. Our results show differences in the uptake of phosphite within and between species, and between season of application and differences in persistence of phosphite in plant stems. For example, in *Banksia grandis* phosphite was detected for 18 months longer in stem tissues after the spring application than after the autumn application. In contrast, there was more phosphite present in the stems of *Leucopogon verticillatus* 2 weeks and 6 months after the autumn than the spring phosphite application. After application of 5 g phosphite/L the highest average concentrations of phosphite measured in the inoculated stems varied between species, from 26 to 299 µg/g dry wt in *L. verticillatus* and *Hibbertia furfuracea*, respectively. Whilst after an application of 10 g phosphite/L the average phosphite levels varied between 41 to 668 µg/g dry wt in *L. verticillatus* and *H. furfuracea*, respectively. This indicates that phosphite uptake varies between species. It is also apparent that the presence or absence of phosphite in the stem is not strictly correlated with the ability of the tissue to inhibit *P. cinnamomi* growth. For example, after the autumn phosphite application to *B. grandis* phosphite was not detected after 12 months, however *P. cinnamomi* growth was controlled for up to 24 months. In contrast, phosphite was still detected in *B. grandis* tissues 24 months after the spring applications of 10 and 20 g phosphite/L, however, *P. cinnamomi* was only contained in plants treated with 20 g phosphite/L. Although phosphite was not detected in *B. grandis* stems 12 months after the autumn phosphite application, it is likely to have still been present but at levels
less than 30-50 µg/g of 0.5 g dry tissue, which is below the detectable range of phosphite concentrations using the HPIC method (Roos et al. 1999).

As the rate of phosphite applied increased so did the concentrations of phosphite in plant tissue and its effectiveness and long term control of P. cinnamomi. However, at 10 and 20 g phosphite/L, phytotoxicity was unacceptably high for some of the plant species. For example, Trymalium ledifolium and Astroloma xerophyllum, which are obligate seeders, were very susceptible to phosphite-induced phytotoxicity and many plants died. Consequently, we do not recommend that rates above 5 g phosphite/L be used. When 5 g phosphite/L was used, it was only effective for between 5 and 12 months. If longer periods of protection are required then some plant deaths will have to be accepted and sprays of 10 or 20 g phosphite/L used. However, if it is considered acceptable that some plants of phosphite sensitive species will be killed or severely burnt, then 20 g phosphite/L can be used which may extend the effectiveness of P. cinnamomi control to 12 and 24 months or beyond in some species. It would be appropriate to conduct research that examined the effectiveness of more than one application of 5g phosphite/L over a short time interval to determine if phytotoxic effects could be by-passed and tissue concentrations of phosphite increased. Alternatively, it would be appropriate to examine other strategies which can increase the uptake of phosphite without phytotoxic effects, such as improved use of surfactants or sticking agents.

Inoculation of stems in this study rather than roots was due to the need to inoculate a large number of plant species without disrupting plant communities by removing plants, and to ensure that the pathogen was not introduced into the soil in the sandplain and jarrah forest sites. So although, P. cinnamomi is generally considered to be a root pathogen (Shearer et al. 1989), it has also been shown to infect collars (Hardy et al. 1996). Consequently, we decided to use stem inoculations for these reasons, and as a result of previous studies which have shown disease development after stem inoculations to correlate well with root inoculations (Shearer et al. 1987b). These observations were also supported by glasshouse trials (Wilkinson et al. 2000 submitted) which demonstrated that the levels of phosphite in the main root of Banksia grandis, Banksia hookeriana and Dryandra sessilis were equivalent or less than the levels in inoculated stems of these species.

Our results clearly indicate that phosphite varies in its effectiveness and persistence between plant species, season of application and rate of application. These differences are a result of the differences in plant uptake of phosphite, susceptibility of the species to phosphite, and environmental factors. Consequently, the frequency of phosphite application and the rates applied to a plant community will need to be based on costs of application and on perceived risks of P. cinnamomi to individual species within a community and the community as a whole. To date many of the risks are not well understood, for example effects on beneficial organisms, plant reproduction and selection for phosphite tolerant P. cinnamomi isolates. However, despite these unknowns, our studies in these plant communities certainly indicate that phosphite has considerable potential to reduce the impact of P. cinnamomi in the immediate future. Although, it must be reiterated that plant species within a community do respond very differently to each other in terms of their responses to phosphite within and between seasons, subsequent phytotoxicity effects, and their ability to contain P. cinnamomi over time. Therefore, it is apparent that phosphite does not give clearly defined outcomes when applied to diverse plant communities.
Recommendations

- Vegetation may be treated with phosphite in spring or autumn.
- A single target species cannot be used to determine the appropriate time for reaplication of phosphite.
- To maintain protection of all plant species tested, phosphite needs to be reapplied every 6-12 months.
- Foliar application of 5 g phosphite/L is recommended to provide protection to plants and to minimise the affects of phytotoxicity.

Recommendations for future research

- To examine biochemical defense mechanisms in different hosts and the influence of in planta levels of phosphite.
- To investigate ways of increasing the uptake of phosphite.
- To develop more sensitive methods of phosphite detection in plant tissues.
- To determine how and where phosphite is moved and stored in the plant with seasonal changes.
- To develop methods that are rapid and cost effective which allow industry and land managers to determine when it is necessary to reapply phosphite to maintain its effectiveness.
2.2 The long-term ability of phosphite to control Phytophthora cinnamomi in five plant species native to Western Australia, in a glasshouse trial

Abstract

Phosphite slowed, but did not completely inhibit colonisation of stems by Phytophthora cinnamomi. For example in Banksia hookeriana, 2 weeks after application of 5 g phosphite/L, P. cinnamomi growth rate was inhibited by 57%, compared to the non-phosphite treated control plants. The longevity of phosphite efficacy varied with different plant species. Foliar application of 5 and 10 g phosphite/L decreased the growth rate of P. cinnamomi in Dryandra sessilis for at least 12 months after it was applied. Application rates of 5 and 10 g phosphite/L was effective in B. grandis and 10 g/L was effective in B. hookeriana for at least 18 months after it was applied. In Hibbertia commutata and Dampiera linearis phosphite was effective for less than 6 and 12 months, respectively. In a second trial, when plants were inoculated with P. cinnamomi at different time periods after phosphite was applied and the time to death was recorded there was a range of responses depending on the plant species and the time of year they were inoculated. The ambient temperature may have affected the efficacy of phosphite. The phosphite levels measured in the stems and roots of B. grandis, B. hookeriana and D. sessilis was initially different in the different species and the rate of decrease of phosphite over time also differed between plant species. Overall, levels of phosphite in stems were higher or equivalent to those in the roots.

Introduction

To increase our knowledge about the long-term effectiveness of phosphite in a range of plant species glasshouse trials were conducted. In the glasshouse, plants can be inoculated and left until death without fear of introducing the pathogen into the native vegetation; the age of all plants within a species can be kept the same, and it is easy to harvest roots for phosphite analysis. Therefore, two studies were conducted to examine the effect of phosphite on (a) the growth rate of P. cinnamomi in plants inoculated at different times after phosphite application and (b) the rate of death of plants inoculated at different times after phosphite application.

Methods

Experiment 1; colonisation of stems by Phytophthora cinnamomi following phosphite application

Experimental design. Five plant species that are representative of common families that occur throughout the south west of Western Australia were used; Banksia grandis Willd. (Proteaceae), Banksia hookeriana Meisn. (Proteaceae), Dryandra sessilis (Knight) Domin. (Proteaceae), Dampiera linearis R.Br. (Goodeniaceae) and Hibbertia commutata Steud. (Dilleniaceae). The experiment was a complete randomised design where plants were sprayed with 0 (control), 5 or 10 g phosphite/L and inoculated with P. cinnamomi at one of five time periods before or after spray application. At each inoculation time, ten plants from each treatment combination were inoculated.
**Plants.** One-year-old plants were initially grown in 150 mm diameter pots which contained equal volumes of peat (Floratorf® 500, Oldenburg, Germany) and coarse yellow sand. The mix was steam pasteurised at 60°C for one hour and every 50 L of mix was supplemented with basal nutrients (O’Gara *et al.* 1996). The plants were potted into 200 mm diameter pots for the inoculations 6 and 12 months after phosphite spray and 290 mm diameter pots for the inoculation after 18 months. Plants were fertilised approximately every 9 months with 0.5 g/pot of Osmocote plus (Scotts Europe BV, Heerlen, The Netherlands), which is low in phosphorus.

Plants were maintained in a tunnelhouse, under 50% shadecloth. However, at the time of the 6-month inoculation the plants were placed in a glasshouse (evaporatively cooled and bar heated) for 2 weeks before and after inoculation. This was to increase the ambient temperature as the inoculation was conducted in winter. The ambient temperatures in the tunnelhouse and glasshouse were monitored throughout the experiments.

**Phosphite application.** Three concentrations of Foli-R-Fos 400 (400g/L phosphorous acid present as mono-di potassium phosphite, Unitec Group Pty Ltd) and 0.25% of the adjuvant Synertrol Oil (Organic Crop Protectants Pty Ltd, NSW) were applied to the plants with backpack spray units in January (summer) 1998. The foliage was sprayed to run-off and the soil in the pots was covered with plastic to prevent phosphite leaching into the soil.

**Inoculum production and plant inoculation.** The *P. cinnamomi* isolate (MP94-48) used in this study was obtained from a diseased *Eucalyptus marginata* in the south west of Western Australia. Plants were inoculated under the bark with *P. cinnamomi* colonised Miracloth discs (6 mm diameter) as an inoculum source. The methods used to prepare and colonise the Miracloth discs have been described by O’Gara *et al.* (1996). The Miracloth discs were colonised with *P. cinnamomi* 10 days prior to inoculation of plants.

The main stem (*Banksia grandis, Banksia hookeriana, Dryandra sessilis*) or lateral branches (*Dampiera linearis, Hibbertia commutata*) were underbark inoculated 20 cm from the base of the plant using the methods of O’Gara *et al.* (1996). Briefly, the plant stem was cut with a scalpel through the periderm to the phloem in an upward movement and a colonised Miracloth disc was inserted into the phloem wound. The wound was then sealed with Parafilm (American National Can™ Chicago IL) to prevent desiccation of *P. cinnamomi*.

Plants were inoculated 2 days prior to phosphite application or 2 weeks or 6, 12 and 18 months after phosphite was applied. Due to low plant numbers, *Hibbertia commutata, Dryandra sessilis,* and *Dampiera linearis* were not included in the first inoculation, *H. commutata* was not included in the 12 or 18 month inoculation and *D. sessilis* was not included in the 18 month inoculation.

The height and diameter (5 cm above the soil level) of *Banksia grandis, Banksia hookeriana* and *Dryandra sessilis* were measured before the plants were sprayed with phosphite and again prior to the plants being inoculated 6, 12 or 18 months after phosphite application, to determine the effect of phosphite on plant growth.

**Colonisation of stems by *P. cinnamomi.*** Plants were harvested 7 to 29 days after they were inoculated depending on plant species, phosphite concentration and visual lesion extension. At harvest, the
inoculated stems were removed, and, depending on the plant species, 18 to 28 cm of the stem above the inoculation point was cut sequentially into 1 cm segments. Each segment was cut in half longitudinally and placed, cut surface down, on a Phytophthora selective ((Shearer et al. 1995), modified by the addition of 10 mg/L Rifampicin (Rifadin, Hoescht Marion Ruessel, Italy)) medium to determine the distance of stem colonised by *P. cinnamomi*, irrespective of the lesion length. The growth rate of *P. cinnamomi* was calculated by dividing the maximum distance that *P. cinnamomi* grew out from the inoculation point by the number of days since the plants were inoculated.

At each harvest root and stem material of *Banksia grandis*, *Banksia hookeriana* and *Dryandra sessilis* were prepared for phosphite/phosphate analysis using High Performance Ion Chromatography (Roos et al. 1999). For each plant species two control plants and six plants treated with each phosphite concentration were analysed at each inoculation time. *B. hookeriana* tissue was not analysed for the inoculation event two days prior to phosphite application or 18 months after phosphite was applied due to the high cost of analysis.

Soil samples were sent to an analytical laboratory (CSBP) to be analysed for total phosphorus (Colwell (Colwell 1963)).

**Statistical analysis.** The *P. cinnamomi* growth rate data for each plant species were analysed using analysis of covariance (ANCOVA) with the independent variables phosphite spray concentration and inoculation time. The growth rate data were log (x + 0.1) or square root transformed to make residuals homoscedastic and approximately normal. Stem diameters, at the point of inoculation, and plant heights (*Banksia grandis*, *Banksia hookeriana* and *Dryandra sessilis*) were included as covariates. Least Squares Means were used (α=0.05) to determine if there was a difference in growth rate of *P. cinnamomi* in plants sprayed with different phosphite concentrations at each inoculation time.

Growth rate and phosphite data were averaged for each phosphite spray concentration at each inoculation time and coefficients of determination (r^2) were used to ascertain if there was a correlation between the phosphite concentration detected in the stems of each plant species and the growth rate of *P. cinnamomi* at each phosphite spray concentration (5 and 10 g phosphite/L). The coefficient of determination was also used to ascertain if there was a correlation between the phosphite detected in plant roots and stems.

Height and diameter increment were calculated for each inoculation time and analysed by ANOVA.

**Experiment 2; plant survival following inoculation of stems of plants treated with phosphite**
The methods used for this trial were the same as those described above unless stated otherwise.

**Experimental design.** The experiment was a complete randomised design with three concentrations of phosphite (0, 5 and 10 g/L) and 0.25% surfactant was applied to run-off to 5 plant species. Plants were inoculated at 3 time periods after phosphite was applied. At each inoculation time ten plants from each treatment combination were inoculated.

**Plant inoculation.** Plants were inoculated in the stem, 10 cm from the base of the plant. All plant species were inoculated 2 days prior to phosphite application or 2 weeks or 7 months after phosphite was applied.
**Plant survival and harvest.** Plant survival was monitored daily and the time to death of the plant was recorded. Plants that did not die after inoculation at the first or second inoculation time were harvested 122 or 106 days after they were inoculated, respectively. The last plant death occurred at least 1 month prior to the live plants being harvested. The plants that were inoculated 7 months after phosphite was applied were harvested 99 days after they were inoculated. When plants died or at harvest 2.5 cm stem segments from either side of the inoculation point and lesion front (if lesions were visible) were cut in half or quarters longitudinally and placed, cut surface down on NARPH medium to recover *P. cinnamomi*.

*Banksia grandis* stems, from the second inoculation period (harvested approximately 4 months after the plants were sprayed), were analysed for phosphite/phosphate using HPIC.

**Results**

**Experiment 1; colonisation of stems by *Phytophthora cinnamomi* following phosphite application**

There was a significant (p<0.05) reduction in the growth rate of *P. cinnamomi* in *Banksia grandis* treated with 5 or 10 g phosphite/L for up to 18 months after phosphite was applied (Figure 2.5). In *Banksia hookeriana*, the growth rate of *P. cinnamomi* was significantly (p<0.05) reduced for 12 months after phosphite was applied. However, only 10 g phosphite/L was effective 18 months after plants were sprayed with phosphite (Figure 2.5). The average inhibition of *P. cinnamomi* growth was only 57 and 67% in *B. hookeriana* when they were inoculated 2 weeks after they were treated with 5 or 10 g phosphite/L, respectively (Table 2.5). Twelve months after inoculation the average inhibition was 56 and 60% for 5 and 10 g/L application, respectively. In *Dampiera linearis* the growth rate of *P. cinnamomi* was only significantly (p<0.05) reduced when they were inoculated immediately after phosphite was applied (Figure 2.5). *P. cinnamomi* colonisation was significantly (p<0.05) reduced in *Dryandra sessilis* for at least 12 months after it was applied. In contrast, there was no control of *P. cinnamomi* growth in *Hibbertia commutata* by 6 months after phosphite application (Figure 2.5).
Figure 2.5. The effect of phosphite on the average growth rate of *Phytophthora cinnamomi* in the stems of five Western Australian native plant species. Plant species were inoculated at different time periods after phosphite was applied. Points with different letters indicate a significant (p<0.05) difference in the growth rate of *P. cinnamomi* within each plant species and time of inoculation (n=10).

The growth rate of *P. cinnamomi* in all plant species not treated with phosphite was slowest for the 6-month inoculation time. The lowest daily minimum (11°C), maximum (32°C) and average maximum (25.5°C) occurred during this inoculation period (Table 2.4). For all other inoculation periods the average maximum temperature was at least 28°C (Table 2.4).
Table 2.4. Minimum and maximum temperatures (°C) between inoculation and harvest in Experiment 1.

<table>
<thead>
<tr>
<th>Inoculation time</th>
<th>Lowest minimum (°C)</th>
<th>Highest minimum (°C)</th>
<th>Average minimum (°C)</th>
<th>Average maximum (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 days prior</td>
<td>16.6</td>
<td>37.6</td>
<td>20.1</td>
<td>30.9</td>
</tr>
<tr>
<td>2 weeks after</td>
<td>13.6</td>
<td>37.6</td>
<td>19.4</td>
<td>30.3</td>
</tr>
<tr>
<td>6 months after</td>
<td>11.0</td>
<td>32.0</td>
<td>17.6</td>
<td>25.5</td>
</tr>
<tr>
<td>12 months after</td>
<td>12.8</td>
<td>38.9</td>
<td>16.9</td>
<td>33.4</td>
</tr>
<tr>
<td>18 months after</td>
<td>15.0</td>
<td>33.0</td>
<td>19.5</td>
<td>28.6</td>
</tr>
</tbody>
</table>

*Time of inoculation relative to phosphite application

Table 2.5. Average inhibition of *Phytophthora cinnamomi* growth in three plant species sprayed with 5 or 10 g phosphite/L and the average phosphite concentration detected in the stems of these plants (n=6).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Inoculation time</th>
<th>Phosphite spray concentration (g/L)</th>
<th>Inhibition (%) ± SE</th>
<th>Stem phosphite concentration (µg/g ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Banksia grandis</em></td>
<td>2 weeks</td>
<td>5</td>
<td>86 ± 3</td>
<td>1284 ± 157</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>92 ± 4</td>
<td>2462 ± 444</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>5</td>
<td>89 ± 3</td>
<td>209 ± 34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>88 ± 3</td>
<td>398 ± 95</td>
</tr>
<tr>
<td><em>Banksia hookeriana</em></td>
<td>2 weeks</td>
<td>5</td>
<td>57 ± 8</td>
<td>1438 ± 245</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>67 ± 10</td>
<td>1674 ± 409</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>5</td>
<td>56 ± 9</td>
<td>563 ± 143</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>60 ± 6</td>
<td>1922 ± 369</td>
</tr>
<tr>
<td><em>Dryandra sessilis</em></td>
<td>2 weeks</td>
<td>5</td>
<td>92 ± 2</td>
<td>2393 ± 308</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>93 ± 1</td>
<td>4083 ± 977</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>5</td>
<td>74 ± 7</td>
<td>635 ± 130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>66 ± 9</td>
<td>1402 ± 440</td>
</tr>
</tbody>
</table>

*Time of inoculation relative to phosphite application

*Inhibition is *P. cinnamomi* growth relative to the control plants

The effectiveness of *P. cinnamomi* inhibition in *Banksia grandis* was not reduced with a decline of tissue phosphite levels of 1284 to 209 µg g⁻¹ dry weight in plants treated with 5 g phosphite/L or from 2462 to 398 µg g⁻¹ dry weight in plants treated with 10 g phosphite/L (Table 2.5). This trend was also observed in *Banksia hookeriana*. Although, in *B. hookeriana* treated with 5 g phosphite/L, the level of phosphite also dropped markedly over 12 months but levels were maintained in plants treated with 10 g phosphite/L. In contrast, as tissue levels of phosphite decreased in *Dryandra sessilis* the inhibition of *P. cinnamomi* also decreased (Table 2.5).

The phosphite levels decreased in the plants over time (Figure 2.6, Table 2.5). Phosphite was detected in the roots and stems for up to 18 months after it was applied in *Banksia grandis* and *Banksia*...
hookeriana and for up to 12 months in Dryandra sessilis (Figure 2.6). There was a significant correlation in the average phosphite concentration in the plant roots and stems for B. grandis ($r^2=0.9$, $p<0.001$) and D. sessilis ($r^2=0.8$, $p=0.02$). However, the correlation was not significant in B. hookeriana ($r^2=0.3$, $p=0.1$). The phosphite levels in the plant stems were higher or equivalent to the phosphite levels in the plant roots for all plant species (Figure 2.6).

Figure 2.6. The average phosphite concentration ($\mu$g g$^{-1}$ dry wt ± SE) in the stems and roots of three Western Australian native plant species at the time of harvest for analysis of Phytophthora cinnamomi colonisation. Plants were foliar sprayed with 0 (n=2), 5 (n=6) or 10 (n=6) g phosphite/L and inoculated at different times before or after this spray. They were harvested 7-29 days after inoculation.

There was a significant correlation ($r^2=0.6$, $p=0.02$) in the average growth rate of P. cinnamomi in the stems of Banksia hookeriana and the average levels of phosphite detected in their stems. In contrast, this correlation was not significant ($p>0.05$) for Banksia grandis or Dryandra sessilis. There was no significant correlation ($p>0.05$) in the average growth rate of P. cinnamomi in the stems of any of the plant species analysed and the average phosphite levels in their roots.

The phosphate concentration in the stems and roots varied greatly between and within each plant species. For example, the phosphate concentration in the stems of Banksia grandis, Banksia hookeriana and Dryandra sessilis ranged between 2.1-9.9, 3.4-22.6 and 5.3-17.1 mg g$^{-1}$ dry tissue weight, respectively (data not shown). The total phosphorus in the soil ranged from 7.9-75 mg P kg$^{-1}$ soil.

Application of phosphite had no significant ($p>0.05$) effect on the height or diameter increment of Banksia grandis, Banksia hookeriana or Dryandra sessilis (data not shown). The average height increments of each plant species 12 months after they were treated with phosphite were B. grandis 67 (SE±4.4) cm, B. hookeriana 46 (SE±3.2) cm and D. sessilis 69 (SE±4.0) cm.
Experiment 2; plant survival following inoculation of stems of plants treated with phosphite

The rate of plant death and the number of plants that died varied between plant species, phosphite spray concentration and inoculation times (Figure 2.7). Very few of the control Banksia grandis or Dampiera linearis died and only one phosphite treated D. linearis died during any of the inoculation periods. When B. grandis or D. linearis were inoculated 7 months after phosphite was applied none of the control or phosphite treated plants died. When Banksia hookeriana were inoculated with P. cinnamomi 2 days prior and 2 weeks after phosphite was applied the plants treated with phosphite did not die as fast as the control plants. When B. hookeriana were inoculated 7 months after phosphite was applied there was only one death of a phosphite treated plant while 90% of the control plants died. The results for Dryandra sessilis were similar to B. hookeriana except more phosphite treated plants died and death was more rapid after the first and second inoculation times. When Hibbertia commutata were inoculated with P. cinnamomi prior to phosphite application there were more deaths of phosphite treated plants than when plants were inoculated 2 weeks after phosphite was applied. The percent of control and phosphite treated H. commutata that died was very similar when plants were inoculated 7 months after phosphite was applied.

Phytophthora cinnamomi was recovered from all plant species when they were harvested at the end of the inoculation periods (Table 2.6). The highest recovery of P. cinnamomi was after the third inoculation (10 months after phosphite application) where P. cinnamomi was isolated from at least 80% of the surviving plants irrespective of phosphite treatment. There was a high recovery of P. cinnamomi (≥80%) from Banksia grandis after all inoculation periods (Table 2.6) even though very few plants were killed (Figure 2.7). For Hibbertia commutata for the first and second inoculation times there was poor recovery (33-57%) of P. cinnamomi from phosphite treated plants (Table 2.6). There was a high recovery of P. cinnamomi from Dampiera linearis that were inoculated two days prior to application of 0 (86%) or 5 g phosphite/L (78%), however there was poor recovery (37%) of P. cinnamomi from plants treated with 10 g phosphite/L. There was also poor recovery of P. cinnamomi from D. linearis that were inoculated 2 weeks after phosphite was applied (50% recovery) irrespective of phosphite treatment. The recovery of P. cinnamomi from Dryandra sessilis after the first and second inoculation periods was variable (33-100%), however only a small number (1-3) of plants that had been treated with phosphite survived.
Table 2.6. The number of plants surviving after they were inoculated with *Phytophthora cinnamomi* at different time periods after phosphite was applied and the percent of live plants from which *P. cinnamomi* was recovered. There were 10 replicates in each treatment.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Spray concentration (g/L)</th>
<th>Inoculation time relative to phosphite application</th>
<th>Plants alive</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 days prior</td>
<td>2 weeks after</td>
<td>7 months after</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plants alive</td>
<td>Plants alive</td>
<td>Plants alive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><em>Banksia grandis</em></td>
<td>0</td>
<td>8</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td><em>Banksia hookeriana</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td><em>Dryandra sessilis</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td><em>Dampiera linearis</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><em>Hibbertia commutata</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

*The plants that were inoculated 2 days prior, 2 weeks and 7 months after phosphite was applied were harvested and recorded as alive/dead 122, 106 or 99 days after they were inoculated, respectively.
- = no plants remaining.
Figure 2.7. Time to death of five Western Australian plant species that were inoculated with *Phytophthora cinnamomi* at three time intervals relative to foliar application of 0, 5 or 10 g phosphite/L. The time to death was grouped into four categories (i) 0-28 days (ii) 29-56 days (iii) 57-84 days (iv) 85-112 days.

The average phosphite levels in *Banksia grandis* that had been inoculated with *P. cinnamomi* 2 weeks after they were sprayed with 5 or 10 g phosphite/L and harvested 4 months after phosphite application were 635 (SE±47) and 1548 (SE±384) µg g⁻¹ dry stem weight, respectively. The phosphate concentration in the stems of these plants ranged between 1.7-9.2 mg g⁻¹ dry stem weight.

The mean daily temperature was higher when plants were inoculated 2 days prior and two weeks after phosphite was applied than when they were inoculated 7 months after phosphite was applied (Figure 2.8).
Figure 2.8. The mean daily temperature for the time the plants were inoculated with *Phytophthora cinnamomi*. The arrows indicate when the plants were inoculated and the plants were harvested at 186 (first and second inoculations) or 349 (third inoculation) Julian days.

**Discussion**

Phosphite substantially decreased the growth rate of *P. cinnamomi* in all plant species when they were infected immediately after phosphite was applied. For some, but not all species this increased resistance persisted. For example, in *Banksia grandis* the reduction of growth of *P. cinnamomi* 12 months after plants were sprayed with phosphite was similar to the initial control of growth immediately after phosphite was applied. On the other hand, in *Dampiera linearis* there was no significant reduction in growth of *P. cinnamomi* in phosphite treated plants 12 months after the plants were inoculated. This is similar to what occurred in the field trials where there was also a range of responses in different plant species that had been treated with phosphite and inoculated with *P. cinnamomi* over time (Chapter 2.1).

The phosphite levels in stems were higher or equal to the levels in the mature roots in all species analysed. As *P. cinnamomi* normally infects the root and the base of the stem of plants, it is the phosphite in the roots that the pathogen would ‘naturally’ encounter. Thus, this result helps support the use of underbark stem inoculation as a suitable bioassay for the levels of protection afforded to the plant by phosphite.

In general, the phosphite levels in the plant stems and roots declined over time. The exception to this was in *Banksia hookerianna* where the phosphite levels in the stem of plants treated with 10 g phosphite/L did not drop until 12-18 months after the plants were treated. The initial levels of phosphite and the rate of decline of phosphite varied markedly between species. The difference in initial levels of phosphite may be explained by differences in retention and uptake due to factors such as orientation, roughness and hydrophobicity of the leaf surface (Ruiter et al. 1990) and differences in leaf structure such as cuticle composition and thickness and the number and position of stomata relative to the leaf surface. There is no evidence that plants are able to metabolise phosphite and therefore decline in levels may be due to dilution through plant growth, leaf fall (Guest et al. 1991) and root exudation (d'Arcy-Lameta et al. 1991), senescence and leakage (Ouimette et al. 1990). Further studies are required to determine what
happens to phosphite within the plant over time and why phosphite levels decrease at different rates in different plant species.

In *Banksia hookeriana* there was a direct relationship between inhibition of *P. cinnamomi* and the phosphite levels in the stem, however in the other plant species analysed there was no significant relationship. For example, in *Banksia grandis* after treatment with 5 g phosphite/L, phosphite levels in the stems 2 and 12 months after application were 1284 and 209 µg/g dry weight, respectively, however, inhibition was 86 and 89%, respectively. Therefore, despite a 6-fold decrease in phosphite levels in the stem inhibition of *P. cinnamomi* remained the same. This indicates that in *B. hookeriana* phosphite is having more of a direct effect on the pathogen whereas in *B. grandis* factors other than phosphite are having more of an effect on the growth rate of *P. cinnamomi*. Further research is required to determine the mode of action of phosphite in different plant species.

Glasshouse grown *Banksia grandis* had higher initial levels of phosphite and retained more phosphite over time than *B. grandis* growing in the jarrah forest. For example, the average phosphite levels in the stems of glasshouse grown *B. grandis* were over 750 and 380 times greater than field grown plants treated in autumn with 5 or 10 g phosphite/L, respectively (Chapter 2.1, Figure 2.2b). Similarly, the levels were over 30 times greater in the glasshouse grown plants than the field grown plants treated with 5 or 10 g phosphite/L in spring (Chapter 2.1, Figure 2.2a). Phosphite treated *Eucalyptus marginata* grown in glasshouse conditions contained 8 times more phosphite in their stems 12 weeks after 5 g phosphite/L was applied than *E. marginata* growing in a rehabilitated bauxite minepit, 6 weeks after phosphite was applied (Chapter 3.3, Table 3.6). It was suggested that high humidity in the glasshouse might accelerate the uptake of phosphite through the stomata and cuticle by slowing the drying time of the chemical (Chapter 3.2). A study by Ouimette and Coffey (1990) showed that phosphite is actively transported into the phloem transport system via a carrier. It is not known if active transport is facilitated by increased humidity. Further studies on the mechanisms of phosphite uptake and the difference in uptake between field and glasshouse grown plants may elucidate ways to increase uptake in the field.

*Phytophthora cinnamomi* killed control plants more rapidly when plants were inoculated in summer than winter. The only measured difference between these inoculation times was the average daily ambient temperatures, which ranged between 20-28°C or 13-20°C for 28 days after the second inoculation and third inoculation events, respectively. Shearer et al. (1987a) found that, in the field, there was a positive relationship between the ambient temperature and the growth of *P. cinnamomi* in the roots of *Banksia grandis* and *Eucalyptus marginata*. The growth rate of *P. cinnamomi* in field inoculated plants has been shown to be adversely affected by low water status in the plant (Bunny et al. 1995). Thus, in glasshouse trials the relationship between the growth of *P. cinnamomi* and ambient temperature may be strengthened, as plants were not drought stressed when the temperatures were high.

We do not know why phosphite was less effective in preventing death of *Banksia hookeriana* and *Dryandra sessilis* when they were inoculated immediately after phosphite was applied, rather than 7 months later. While the effect of temperature on the growth of *P. cinnamomi* is fairly well understood there has been no studies on the effect of temperature of the efficacy of phosphite. *Hibbertia commutata* was the only species where phosphite was not as effective for the 6-month inoculation event. This indicates that in *H. commutata*, *P. cinnamomi* is able to overcome plant defense and/or phosphite levels were not high enough to inhibit *P. cinnamomi* growth whereas in the other species the combination of
phosphite and plant defense were able to inhibit the growth of *P. cinnamomi*. It is interesting that *P. cinnamomi* was recovered from almost all plants following the 6-month inoculation. This shows that *P. cinnamomi* was still alive but for some reason unable to grow. Further research is needed to determine if the ambient temperature affects the efficacy of phosphite.

*Phytophthora cinnamomi* was recovered from a substantial number of phosphite treated plants that had not died during the experiment. Other researchers have also found that *in planta* phosphite inhibits the growth of *Phytophthora* spp. but does not kill them (Marks *et al.* 1990; Marks *et al.* 1992; Ali *et al.* 1998; Ali *et al.* 1999). The consequence of the continued presence of *P. cinnamomi* is that once phosphite levels have dropped below the levels required to contain the pathogen plant death may still occur unless phosphite is reapplied.

*Phytophthora cinnamomi* caused very few deaths of control *B. grandis* irrespective of the time of year the plants were inoculated. The high recovery of *P. cinnamomi* from all control plants shows that the pathogen was alive but contained within the site of inoculation. This conflicts with Shearer *et al.* (1988) who found that once *B. grandis* are infected with *P. cinnamomi* they show no evidence of host resistance. While extensive lesions were observed on a number of the control *B. grandis* stems, in our trials, the plants were able to wall-off the pathogen before they were killed. It is unlikely that the *P. cinnamomi* isolate was not pathogenic on *B. grandis* as this isolate killed up to 90% of underbark inoculated plants in the field (Chapter 2.2, Table 2.9). It is possible that *B. grandis* grown in relatively high nutrient soil and with adequate water availability are able to respond to invasion by *P. cinnamomi* differently from plants under forest conditions of low soil fertility.

We were unable to grow plants of the same low phosphorus status in the glasshouse as found in field soils, even though the potting mix used contained low levels of available P. Western Australian native plants are able to grow in low nutrient soils as they have strategies to scavenge nutrients efficiently (Lamont *et al.* 1982). As the plants in the current experiments were fertilised with low P slow release fertiliser it is likely that they were efficient at scavenging the available P from the potting mix. The effect of high plant phosphate levels on the efficacy of phosphite is an area that needs further research.

The results for these glasshouse trials have confirmed some important findings in native plant communities: (i) phosphite levels decrease in the plants over time, (ii) there is a gross relationship between the phosphite levels in the plant and control of *P. cinnamomi* growth, (iii) there is a considerable difference between species both in their response to phosphite and *P. cinnamomi*, and (iv) there is a difference in growth rates of *P. cinnamomi* when plants are inoculated at different times of year (Chapter 2.1).

Further work is required to identify factors that contributed to the different plant responses in the two glasshouse trials, particularly the efficacy of phosphite in *Dryandra sessilis* and *Banksia hookeriana*. The differences observed in the growth of *P. cinnamomi* colonisation and the efficacy of phosphite at different times of the year highlight the need to explore mechanisms of phosphite action under control conditions.
Recommendations

- A single target species cannot be used to determine the time for the reapplication of phosphite.
- If underbark inoculation is to be used as a bioassay for the levels of induced resistance from phosphite application, plants must be inoculated at a time of year when they are susceptible to *P. cinnamomi*.

Recommendations for future research

- The mechanisms of phosphite uptake by different plant species need to be determined.
- The effects of temperature on the efficacy of phosphite both *in vitro* and *in planta* need to be determined.
2.3 The long-term prevention of death of jarrah forest plant species treated with phosphite and inoculated with *Phytophthora cinnamomi*

**Abstract**

Foliar application of 5 or 10 g phosphite/L was effective in preventing the death of *Banksia grandis* when they were inoculated with *Phytophthora cinnamomi* for at least 9 months after application. Wound and soil inoculation of *Adenanthos barbigera*, *Allocasuarina* sp. or *Grevillea wilsonii* with *P. cinnamomi* killed only one control *A. barbigera*. Therefore, the efficacy of phosphite could not be determined for these species. Application of 5 or 10 g phosphite/L caused severe phytotoxicity and death of *Trymalium ledifolium* plants. However, 22 months after phosphite was applied there was no difference in the number of deaths of *T. ledifolium* treated with 5 g phosphite/L and the controls but almost all of the plants treated with 10 g phosphite/L (97%) had died.

**Introduction**

In *P. cinnamomi* infested areas, when environmental conditions are favourable for *P. cinnamomi* vegetative growth and zoospore production, plants would be constantly challenged by the pathogen. In previous field trials, we have inoculated a plant stem once and subsequently harvested this inoculated stem to determine the growth rate of *P. cinnamomi* in plants sprayed with different phosphite concentrations. This ‘bioassay’ has allowed us to compare the effectiveness of phosphite in containing *P. cinnamomi* in a range of different plant species under different environmental conditions. However, it is also important to know if phosphite is able to protect plants that are growing in a *P. cinnamomi* infested area and thus, are potentially being challenged continuously by the pathogen over time. Therefore, the aim of this study was to determine the long-term ability of phosphite to control colonisation and to protect plants that are continually being challenged by *P. cinnamomi*.

**Methods**

**Trial design and plant species** The experiment was a split plot design established in *E. marginata* forest adjacent to the Alcoa World Alumina Australia Jarrahdale mine (approximately 50 km south-east of Perth). The trial site consisted of 6 blocks each containing 3 main plots that were inoculated 7 days prior or 9 or 18 months after phosphite was applied. Within each main plot there were 5 plant species; *Adenanthos barbigera* Lindl., *Allocasuarina* sp., *Banksia grandis* Wild and *Grevillea wilsonii* A.Cunn and *Trymalium ledifolium* Fenzl Ten plants of each plant species that had been sprayed with 0 (control), 5 or 10 g phosphite/L were inoculated at each time period before or after phosphite was applied.

**Phosphite application** In November (summer) 1997 phosphite (Foli-R-Fos 400, Unitec Group Pty Ltd) and 0.25% Synertrol Oil (Organic Crop Protectants) was applied to run-off to the plants using backpack spray units. Due to low numbers of *Allocasuarina* sp. and *G. wilsonii* the 10 g phosphite/L treatment was not included for these species. All plants treated were between 0.5 and 1.5m high.
Inoculum production and plant inoculation  

Trymalium ledifolium were severely affected by phytotoxicity at both phosphite rates and consequently were not inoculated with P. cinnamomi. Plants were inoculated using the methods described previously (Chapter 2.1). Miracloth discs colonised with P. cinnamomi isolate MP94-48 were used to inoculate the plant stems. Banksia grandis and the Allocasuarina sp. (species with larger diameter stems) were inoculated with 1.5 cm diameter discs while Adenanthos barbigera and Grevillea wilsonii (species with smaller diameter stems) were inoculated with 6 mm diameter discs. Stems of A. barbigera were inoculated 10 cm above the ground level and the other species were inoculated 15 cm above the ground level. B. grandis stems colonised with P. cinnamomi were buried in the soil surrounding the plants as an additional source of inoculum. The colonised B. grandis stems (1-2 cm diameter) were prepared by cutting stems into 2 cm sections, soaking them in deionised water for 12-24 hours, decanting the water and then autoclaving the stem pieces on three consecutive days at 121°C and inoculating them with P. cinnamomi colonised V8 agar. The P. cinnamomi inoculated stem pieces were incubated at 24°C, in the dark for approximately 2 months before they were placed in the soil surrounding the plants. Four B. grandis stem pieces (Banksia plugs) were buried at an equal distance around the plant, approximately 15 cm deep and 25 cm from the plant stem, this was repeated monthly for 4 months following the second and third inoculation times (Table 2.7).

Table 2.7 Schedule for the inoculation of Allocasuarina sp., Banksia grandis, Grevillea wilsonii and Adenanthos barbigera with Phytophthora cinnamomi isolate MP94-48

<table>
<thead>
<tr>
<th>Time relative to phosphite application</th>
<th>Year of inoculation</th>
<th>Number of stem inoculations</th>
<th>Time of stem inoculation</th>
<th>Number of soil inoculations</th>
<th>Time of soil inoculation</th>
<th>Time of monitoring after inoculation (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days prior</td>
<td>1997</td>
<td>1</td>
<td>Nov</td>
<td>1</td>
<td>Nov</td>
<td>25</td>
</tr>
<tr>
<td>18 months</td>
<td>1999</td>
<td>2</td>
<td>May, July</td>
<td>4</td>
<td>May, June, July</td>
<td>8</td>
</tr>
</tbody>
</table>

*B. grandis and Allocasuarina sp. stems were only inoculated once, at the earliest month.*

Plants were first inoculated 7 days prior to or 9 or 18 months after phosphite was applied (Table 2.7). Due to poor symptom development after the first inoculation time an extra stem on each plant of the multi-stemmed species (Adenanthos barbigera and Grevillea wilsonii) was inoculated 2 months after the second and third inoculation times. Plants were monitored every month for death of inoculated stems or whole plants. Trymalium ledifolium were monitored regularly for death due to phytotoxicity.

Five extra control plants of each species were underbark inoculated and Banksia plugs were buried around each plant for the first and second inoculation times. These control plants and the Banksia plugs were harvested 9 months after the first inoculation time to determine if P. cinnamomi was still viable. For the second inoculation time one Banksia plug surrounding each control plant was harvested 2, 7 and 12 months after the first batch of plugs was buried and the percentage recovery of P. cinnamomi recorded. For the second inoculation time the control plants were harvested 12 months after the single...
stemmed species were inoculated and 10 months after the final inoculation of the multi-stemmed species. To harvest the control plants, the inoculated stem was removed from the plant at the base of the stem and the bark around the inoculation point was scraped off the stem to determine if there was a lesion. The inoculation point and the lesioned portion of the stem (if present) was plated onto selective medium using the methods described previously (Chapter 2.1, Experiment 2). Recovery of *P. cinnamomi* was recorded.

**Results**

*Trymalium ledifolium* sprayed with 5 or 10 g phosphite/L died at a faster rate than the control plants (Table 2.8). Twenty-two months after phosphite was applied there was no difference in the percent of deaths of *T. ledifolium* plants that had been treated with 0 (63%) or 5 (67%) g phosphite/L. However, almost all of the plants which had been sprayed with 10 g phosphite/L were dead.

**Table 2.8. Death of *Trymalium ledifolium* after foliar application of 0, 5 or 10 g phosphite/L in November 1997. Final date of monitoring was 22 months after phosphite was applied.**

<table>
<thead>
<tr>
<th>Phosphite concentration (g/L)</th>
<th>1998</th>
<th>1999</th>
<th>Date Monitored</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jan</td>
<td>Mar</td>
<td>June</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>33</td>
<td>39</td>
</tr>
<tr>
<td>10</td>
<td>53</td>
<td>83</td>
<td>89</td>
</tr>
</tbody>
</table>

*0 g phosphite/L n=41; 5 and 10 g phosphite/L n=36
Table 2.9. The effect of foliar application of 0, 5 or 10 g phosphite/L on the survival of plants which have been inoculated with *Phytophthora cinnamomi*.

<table>
<thead>
<tr>
<th>Plant species(^a)</th>
<th>Inoculation time(^b)</th>
<th>Phosphite concentration (g/L)</th>
<th>Inoculated stems dead(^c)(^d) (%)</th>
<th>Plants dead(^d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Adenanthos barbigera</em></td>
<td>7 days prior</td>
<td>0</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9 months after</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>18 months after</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Banksia grandis</em></td>
<td>7 days prior</td>
<td>0</td>
<td>-</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>9 months after</td>
<td>0</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>18 months after</td>
<td>0</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td><em>Grevillea wilsonii</em></td>
<td>7 days prior</td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9 months after</td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>18 months after</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

\(\text{\(^a\) no Allocasuarina sp. died}\)

\(\text{\(^b\) relative to phosphite application}\)

\(\text{\(^c\) no result for plant species with only one main stem}\)

\(\text{\(^d\) plants which were inoculated 7 weeks prior to phosphite application were monitored for 25 months post inoculation while plants inoculated 9 or 12 months after phosphite was applied were monitored for 9 or 12 months post inoculation.}\)

There were few deaths of the inoculated stem or whole plant at all phosphite concentrations (including controls) for all plant species except *Banksia grandis* (Table 2.9). Twenty-four months after
the first inoculation time of *B. grandis* 90, 10 and 10% of the plants treated with 0, 5 or 10 g phosphite/L, respectively, were dead. When *B. grandis* were inoculated 9 months after phosphite was applied 50, 0 and 10% of the plants treated with 0, 5 or 10 g phosphite/L, respectively, were dead. The 18-month inoculation of *B. grandis* was not as successful with only 20% of the control plants dying, however none of the phosphite treated plants died. For *Adenanthos barbigera* and *Grevillea wilsonii* there were no large differences in the number of deaths of the inoculated stems of phosphite treated plants versus the control plants. There was only one death of a plant (control) of *A. barbigera* and no *G. wilsonii*, or *Allocasuarina* sp. died.

When the extra control plants of each species for the first inoculation time were harvested 9 months after the plants were inoculated no *P. cinnamomi* was recovered. Also, *P. cinnamomi* was not recovered from the *Banksia* plugs surrounding these plants.

When the *Banksia* plugs were harvested 1-2 months after they were buried in the ground after the 9-month inoculation time, *P. cinnamomi* was recovered from all of them (Table 2.10). However, when the plugs were harvested in March (4 months later) the recovery of *P. cinnamomi* ranged from 67-90% depending on the time the *Banksia* plugs had been buried (Table 2.10). When the *Banksia* plugs were removed from the ground in July (10-12 months after they were buried) most of them could not be recovered because they had been eaten by termites. However, *P. cinnamomi* was isolated from at least one *Banksia* plug surrounding each plant. When the spare control plants were harvested in July, 10 months after the multi-stemmed and 12 months after the single stemmed plants were last inoculated, no *P. cinnamomi* was recovered.

<table>
<thead>
<tr>
<th>Table 2.10. The recovery of <em>Phytophthora cinnamomi</em> from buried colonised <em>Banksia</em> stem pieces.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Month buried</strong></td>
</tr>
<tr>
<td>August</td>
</tr>
<tr>
<td>September</td>
</tr>
<tr>
<td>October</td>
</tr>
<tr>
<td>November</td>
</tr>
<tr>
<td>NS= not sampled</td>
</tr>
</tbody>
</table>

**Discussion**

Phosphite prevented the death of *Banksia grandis* for at least 9 months after application. The effectiveness of the treatment after 18 months could not be determined, as plants need to be monitored for a longer period of time. As there was only one death of a non-phosphite treated but inoculated plant of any of the other plant species tested, no conclusions can be made about the longevity or efficacy of phosphite in these species. Application of phosphite to *Adenanthos barbigera* or *Grevillea wilsonii* did
not decrease the death of inoculated stems however, very few control stems died and death of the inoculated stem of phosphite treated or control plants did not lead to death of the plant.

*Phytophthora cinnamomi* was not isolated from the control plants when they were harvested 9 or 10-12 months after they were inoculated after the first and second inoculation times, respectively. This indicates that *P. cinnamomi* did not survive in the plants. The presence of lesions in the plants indicates that *P. cinnamomi* did infect the plants but they were able to wall off the pathogen and were thus protected.

*Trymalium ledifolium* was sensitive to all phosphite concentrations, including the recommended rate (5 g/L). However, 67% of plants not sprayed with phosphite also died over the monitoring period which indicates that this species may be sensitive to drought. Application of 5 g phosphite/L in November appeared to stress the plants during the first summer which caused an increased number of deaths. After the first summer there were more deaths of the control plants at each monitoring time than of plants treated with 5 g phosphite/L. This result indicates that *T. ledifolium* should be sprayed earlier in the year (early spring) to allow the plants time to recover from the effects of phytotoxicity before they are stressed by summer water deficit. Application of 5-20 g phosphite/L in spring (November) and 10-20 g phosphite/L in autumn (May) also caused severe phytotoxicity and death of *T. ledifolium* growing at Huntly (Chapter 2.1, Table 2.3). Interestingly, the same number of controls and plants treated with 5 g phosphite/L died when phosphite was applied in autumn at Huntly but when phosphite was applied in spring 37% more plants treated with 5 g phosphite/L died than controls (Chapter 2.1, Table 2.3). This result also supports the application of phosphite earlier in the year, before summer.

Due to the lack of deaths of control plants no conclusions can be made about the efficacy of phosphite in controlling the colonisation of *P. cinnamomi* or preventing the infection of plants that are continually being challenged by the pathogen. In future it may be more effective to spray plants in a naturally infested area of jarrah forest and monitor deaths of phosphite treated plants over time. This method has been used effectively in areas of more dense vegetation in the sandplain and heath and thicket plant communities of western Australia (*Shearer et al. 1997a; Shearer et al. 1997b; Komorek et al. 1998; Barrett 1999*).

**Recommendations**

- Vegetation that is sensitive to phytotoxicity caused by phosphite should be sprayed earlier than November to avoid drought stress and allow time for them to recover before summer.

**Recommendations for future research**

- To determine the long-term efficacy of phosphite in preventing death of plants that are continually being challenged by *P. cinnamomi*, experimental plots should be set up in naturally infested areas.
Chapter 3

Biology of phosphite-Phytophthora cinnamomi and host plant interactions

The purposes of the following Chapter (3.1-3.3) were to determine:

1. The sensitivity of Phytophthora cinnamomi isolates to phosphite in planta

One of the benefits of phosphite as a fungicide is that repeated applications do not appear to induce tolerance in Phytophthora isolates (Guest et al. 1991). The majority of studies that have examined the sensitivity of Phytophthora species to phosphite have been conducted in vitro. The few in planta studies have used mutated isolates tolerant to phosphite. The use of mutant isolates has substantial drawbacks as in addition to tolerance to phosphite other factors such as pathogenicity or aggressiveness and long-term survival may have changed. Earlier studies in this laboratory (Wilkinson et al. in press-b) have shown that there is a range of sensitivity amongst naturally occurring isolates in vitro. The aim of the present study was to determine whether there is a correlation between sensitivity of isolates to phosphite in vitro and in planta. If there is a good correlation, the growth of an isolate on phosphite in vitro could be used to monitor changes in phosphite sensitivity of isolates to determine if there are differences over time, when a plant community is repeatedly sprayed.

2. If phosphite can inhibit the production of sporangia and release of zoospores from P. cinnamomi infected but contained areas of colonisation in treated plants.

A number of in vitro studies have shown that phosphite inhibits the production of sporangia and zoospores from a range of Phytophthora species. However, there have been no studies that examine these characteristics in planta. It is important to determine if recommended rates of phosphite can inhibit sporangial production from infected plants treated with the fungicide, since zoospores are the major disseminating propagule of Phytophthora. Whether or not phosphite is able to inhibit the production of sporangia and zoospores from infected but contained plant tissues, will determine management procedures in infected areas. This study (Chapter 3.3) was undertaken to determine if phosphite at 5 and 10 g/L phosphite could inhibit the production of sporangia and zoospores from P. cinnamomi infected and phosphite treated plants in the glasshouse and a rehabilitated minesite.

3. The efficacy of phosphite in reducing the infection and colonisation of the roots of a range of native plant species inoculated with Phytophthora cinnamomi.

The majority of our studies (Chapters 2 and 3.1-3.3) have used the stem underbark inoculation with P. cinnamomi to determine the effectiveness and long-term efficacy of phosphite. In addition, a number of our studies (Chapters 2.1 and 3.2) we could not risk the introduction of P. cinnamomi into soil for reasons
of hygiene and due to the difficulty of inoculating roots under field conditions. Therefore, this study (Chapter 3.4) was undertaken to determine:

a) if phosphite could control infection and subsequent colonisation by *P. cinnamomi* zoospores in the roots of a range of plant species under controlled glasshouse conditions, and

b) to confirm that observations made by underbark stem inoculations are similar to those made by root inoculations with zoospores.
3.1 Sensitivity of *Phytophthora cinnamomi* isolates to phosphite *in planta*

**Abstract**

There was no correlation between the sensitivity of *Phytophthora cinnamomi* isolates to phosphite *in vitro* and the ability of phosphite to reduce deaths of stems or entire plants that had been wound inoculated with *P. cinnamomi*. Isolate MP62, which was sensitive *in vitro*, was the least pathogenic isolate and was also the least inhibited by phosphite over the 800-day monitoring period. Isolate MP125, which was more tolerant *in vitro*, caused the greatest number of stem and plant deaths of plants treated with 5 g phosphite/L over the 800 day monitoring period. There was an increase in the number of deaths in plants treated with 5 g phosphite/L 475 days after they were inoculated. This indicates that phosphite controlled the growth but did not kill *P. cinnamomi*. Isolate MP94-03, which was sensitive to phosphite *in vitro*, killed the greatest number of inoculated stems and plants up to 475 days after phosphite was applied.

**Introduction**

Phosphite is now used in Western Australia to control *Phytophthora cinnamomi* on small areas of vegetation with high conservation value (Komorek et al. 1997). Mining companies hope to use phosphite to control *Phytophthora* in rehabilitated areas and on small-infested areas near the mines. It is not known whether the continual use of phosphite in these plant communities will select for phosphite tolerant isolates of *P. cinnamomi* or if isolates will become tolerant to phosphite over time.

A large number of *P. cinnamomi* isolates from areas of the northern jarrah forest of Western Australia (not previously sprayed with phosphite) have been tested *in vitro* to determine the variation in phosphite sensitivity between these isolates (Wilkinson et al. in press-b). The EC$_{50}$ values for these isolates ranged from 4 to 148 µg phosphite/mL.

This trial was designed to determine if (a) *P. cinnamomi* isolates show a range of sensitivities to phosphite *in planta* and (b) if there is a correlation between the *in vitro* and *in planta* tolerance of isolates to phosphite.

**Methods**

**Experimental design** The experiment was a split block design established on a 1 year-old rehabilitated bauxite minepit in jarrah forest of Western Australia (Alcoa World Alumina Australia, Jarrahdale mine). There were five blocks and each block contained three main plots (approximately 15m x 15m) which were sprayed with 0 (control), 5 or 10 g phosphite/L. Within each plot, 20 *Eucalyptus marginata* plants were inoculated with one of five *P. cinnamomi* isolates.
**Phytophthora cinnamomi** isolates, plant inoculation and phosphite application  Five *P. cinnamomi* isolates, which ranged in sensitivity to phosphite *in vitro* (Wilkinson *et al.* in press-b), were used. The isolates consisted of two tolerant (MP97, MP125), two sensitive (MP62, MP94-03) and one moderately tolerant isolate (MP94-48). Plants were underbark inoculated 30-40 cm from the plant lignotuber using the methods described previously (Chapter 2.1). The plants were inoculated in October 1997 (spring) and 1 week later their foliage was sprayed to run-off with Foli-R-Fos 400 (and 0.25% Synertrol Oil) using backpack spray units. Plants were monitored every 1 - 2 months for death of the inoculated stem and the plant. Plant heights were recorded at the time of inoculation, 160 days (autumn) and 400 days (spring) later and the height increment from the time of inoculation was calculated. Stem diameters 25 cm above the ground were measured 160 and 400 days after the plants were inoculated and the diameter increment between these two time periods was calculated.

**Results**

Application of 5 or 10 g phosphite/L slowed the rate of death of inoculated stems and plants in *E. marginata* inoculated with the different isolates of *P. cinnamomi*, compared with plants not treated with phosphite (Figure 3.1). There was a marked increase in the number (13 plants) of inoculated stem deaths 472-600 days after plants were sprayed with 5 g phosphite/L. There was also an increase in the number of deaths of plants treated with 5 g phosphite/L during this time and in the number of inoculated stem deaths (5 plants) of plants sprayed with 10 g phosphite/L. This indicates that the effect of phosphite was wearing off and that *P. cinnamomi* had not been killed by the phosphite treatment.

In plants not treated with phosphite, isolates MP62, MP125, MP94-48, MP94-03 and MP97 killed 4, 10, 12, 12 and 13 of the 20 plants with inoculated stems, respectively (Figure 3.1a). When plants were treated with 5 g phosphite/L isolate MP62 killed more inoculated stems (5) than it did in plants not treated with phosphite, however deaths of the stems of phosphite treated plants started 465 days after they were inoculated (Figure 3.1a). Isolate MP125 killed the largest number (7) of inoculated stems of plants that had been treated with 5 g phosphite/L. The time to death and the number of deaths of the inoculated stems of plants treated with 10 g phosphite/L and inoculated with MP62 was similar to plants not treated with phosphite. For all other isolates the number of deaths of inoculated stems was lower in phosphite treated plants (2-6) than control plants (10-13).

There were very few deaths of control plants (2-6), irrespective of the isolate with which they were inoculated (Figure 3.1b). Isolate MP97 killed the most (6) plants not treated with phosphite and MP62 killed the least (2). Isolate MP125 killed the most plants (5) that had been treated with 5 g phosphite/L and isolates MP62, MP94-03 and MP94-48 killed the least (3). At the last monitoring time (800 days after plants were treated) there was a similar number of deaths of plants treated with 5 g phosphite/L (3-4) as there were in the controls. However, the time to death was generally longer in the phosphite treated plants. Only one plant which was treated with 10 g phosphite/L died and this plant was inoculated with isolate MP62.
Figure 3.1. The effect of phosphite on time to death of (a) the inoculated stem of *Eucalyptus marginata* seedlings or (b) the whole plant in plants that have been inoculated with different *Phytophthora cinnamomi* isolates that have a range of sensitivities to phosphite *in vitro* (n=20). *In vitro* phosphite (i) sensitive isolates MP62 ν and MP94-03 λ, (ii) tolerant isolates MP97 σ and MP125 υ and (iii) intermediate isolate MP94-48 ϒ.

It is not possible to differentiate between the effect of phosphite and *P. cinnamomi* isolate on plant growth as there were no control plants that were sprayed with phosphite but not inoculated with *P. cinnamomi*. Plants inoculated with isolate MP62 and not sprayed with phosphite grew more than the plants treated with phosphite and inoculated with the same isolate (Figure 3.2a and b). Plants inoculated with the other 4 isolates and not treated with phosphite grew less or a similar amount as plants treated with phosphite. The results were similar for diameter growth (Figure 3.2c).
Figure 3.2. The effect of 0, 5 or 10 g phosphite/L on the average growth of *Eucalyptus marginata* seedlings that have been inoculated with one of 5 *P. cinnamomi* isolates. Height growth was measured (a) 160 and (b) 400 days after plants were inoculated and (c) diameter growth of the plant stem 25 cm above the lignotuber 160-400 days after plants were inoculated (mean ± SE, n= a maximum of 20).

**Discussion**

It is difficult to interpret the results for tolerance of isolates to phosphite, as there were insufficient deaths of phosphite treated plants or deaths of their inoculated stems. However, there was no obvious correlation between the tolerance of *P. cinnamomi* isolates to phosphite *in vitro* and *in planta*. While it is difficult to determine definitively which isolates were more tolerant to phosphite *in planta* there was no grouping of isolates into the same sensitive and tolerant categories that had been determined *in vitro* (Wilkinson *et al.* in press-b).

Phosphite did not kill *P. cinnamomi* in the host tissue during the experiment as indicated by the marked increase in the number of inoculated stems that died 472-600 days after the plants were sprayed with 5 g phosphite/L. In a similar experiment (Chapter 3.2, Table 3.4) phosphite was detected in seedling *E. marginata* stems 41 weeks (287 days) after foliar application of 5 or 10 g phosphite/L but was not detected 58 weeks (405 days) after. Thus, in the current experiment the phosphite levels in the plant may have dropped below the levels required to contain *P. cinnamomi* growth.
There are four ways that phosphite tolerance could be determined using the results of this trial; (i) the isolate that caused the greatest number of deaths of treated plants before phosphite levels were likely to have decreased below levels required to inhibit *P. cinnamomi* (ii) the isolate that caused the largest number of deaths over the entire monitoring period (iii) the isolate that was least inhibited in phosphite treated plants before phosphite levels were likely to have decreased below levels required to inhibit *P. cinnamomi* or (iv) the isolate that was least inhibited over the entire monitoring period. Isolates MP62, MP125 or MP94-03 may be classified as more tolerant depending on which of the methods is used.

Control plants inoculated with isolate MP62 grew faster than plants treated with phosphite and inoculated with MP62. To determine if this was a result of MP62 stimulating plant growth, phosphite inhibiting growth or a combination of isolate MP62 and phosphite inhibiting plant growth, non-inoculated phosphite treated (0, 5 or 10 g/L) ‘controls’ were needed. In glasshouse experiments, we have determined that phosphite does not affect the height or diameter growth of *Banksia grandis*, *Banksia hookeriana* or *Dryandra sessilis* (Chapter 2.2) or *Eucalyptus marginata* grown on a rehabilitated bauxite minepit (Chapter 3.2). Therefore, it was likely that either the *P. cinnamomi* isolate MP62 stimulated the growth of non-treated plants or there was a more complex interaction between MP62 and phosphite that adversely affected the growth of phosphite treated plants.

The small number of deaths of phosphite treated plants and the length of time between phosphite application and the majority of deaths meant that no firm conclusions can be made about the phosphite tolerance of the 5 isolates *in planta*. However, there was no evidence for a correlation of *in vitro* and *in planta* sensitivity of *P. cinnamomi* to phosphite. Our work showed that in phosphite treated plants *P. cinnamomi* is initially contained but not killed. Thus, over time, probably due to a drop in phosphite levels, *P. cinnamomi* is no longer contained and may kill the plant.
3.2 Sensitivity of Phytophthora cinnamomi Isolates to Phosphite In Planta

(submitted to Plant Disease for publication)
C. J. Wilkinson, J. M. Holmes, B. Dell, K. M. Tynan and J. A. McComb, I. J. Colquhoun and G. E. St J. Hardy

ABSTRACT

Phytophthora cinnamomi isolates, with a range of sensitivities to phosphite in vitro, were inoculated into plants that had been sprayed with phosphite to determine if there was a correlation between in vitro and in planta sensitivity. Banksia hookeriana and Eucalyptus marginata seedlings, growing in a glasshouse were treated with 0 or 5 g phosphite/L and inoculated three months later with one of 12 P. cinnamomi isolates. There was no correlation between the in vitro and in planta sensitivities of the isolates to phosphite and there was no difference in the percent growth inhibition of the isolates. In a trial on a rehabilitated minesite, E. marginata seedlings were sprayed with 0, 5 or 10 g phosphite/L and inoculated with 5 P. cinnamomi isolates 2, 35 and 52 weeks after treatment. One isolate (MP125) was less inhibited by phosphite in planta in the 2-week phosphite treated plants but there was no difference in the percent growth inhibition of the remaining four isolates. Further, phosphite did not decrease P. cinnamomi growth in plants which were inoculated 35 or 52 weeks after application. The phosphite concentration in the stems of the glasshouse plants was 8 times higher than in the plants growing on the minesite.

INTRODUCTION

Phosphite has been used successfully on horticultural crops to control disease caused by Phytophthora spp. (Flett et al. 1990; Wicks et al. 1990; Greenhalgh et al. 1994; Guest et al. 1995). It is also effective in controlling Phytophthora cinnamomi in a range of native Australian plant species (Shearer et al. 1991; Komorek et al. 1997; Ali et al. 1998; Pilbeam et al. in press). Phosphite is applied to plants by either trunk injection, foliar spray or soil drench. Once it has entered the plant symplast, it can move from the shoots to the roots in the phloem and from the roots to the shoots in the xylem (Cohen et al. 1986).

There are two main hypotheses in the literature about the in planta mode of action of phosphite. Firstly, phosphite acts directly on Phytophthora by inhibiting growth (Coffey et al. 1984; Coffey et al. 1985; Fenn et al. 1985; Rohrbach et al. 1985; Dolan et al. 1988; Coffey et al. 1989; Fenn et al. 1989; Ouimette et al. 1989). Alternatively, phosphite affects the cell wall composition of Phytophthora species causing overproduction of elicitors or a reduction in the production of suppressors. This may lead to an enhanced rate of recognition by the plant to the presence of the fungus and a subsequent increase in expression of plant defense mechanisms (Afek et al. 1989; d'Arcy-Lameta et al. 1989; Griffith et al. 1989a; Dunstan et al. 1990; Guest et al. 1990; Barchietto et al. 1992).
Which hypothesis is supported may be determined by the plant’s defense mechanisms. If the plant being treated has active defense mechanisms, then the concentration of phosphite required \textit{in planta} to inhibit fungal growth may be lower than that required \textit{in vitro}. If the plant’s defense mechanisms are poor then the concentration of phosphite required \textit{in planta} may be as high as the concentration required \textit{in vitro} (Smillie et al. 1989). In \textit{Eucalyptus marginata} Sm., there was an increase in defense enzymes when phosphite levels within the roots were low but not when phosphite levels were high (Jackson et al. in press). This suggests that when plants are treated with high levels of phosphite, the fungus may be inhibited before plant defense mechanisms are stimulated (Griffith et al. 1989c; Smillie et al. 1989; Dunstan et al. 1990).

Phosphite is now used in Western Australia to control \textit{Phytophthora cinnamomi} Rands on small areas of vegetation with high conservation value (Komorek et al. 1997). Mining companies hope to use phosphite to control \textit{Phytophthora} in rehabilitated areas and on small infested areas near the mines. It is not known if the continual use of phosphite in these plant communities will select for phosphite tolerant isolates of \textit{P. cinnamomi} or if isolates will become tolerant to phosphite over time. A large number of \textit{P. cinnamomi} isolates from the northern jarrah forest of Western Australia which has not been treated with phosphite have been tested \textit{in vitro} for their sensitivity to phosphite. The EC\textsubscript{50} values for these isolates ranged from 4 to 148 µg phosphite/mL (Wilkinson et al. in press-b).

The aim of these experiments was to determine if there is a correlation between \textit{in vitro} and \textit{in planta} phosphite sensitivity of \textit{P. cinnamomi} isolates.

**MATERIALS AND METHODS**

**Glasshouse**

\textit{Trial design.} The experiment was a $2 \times 2 \times 12$ factorial in a randomised complete block design. There were 5 replicate blocks, each of which contained one plant of each treatment combination. Treatments were phosphite concentration (0 and 5 g phosphite/L), plant species (\textit{Banksia hookeriana} Meisn. and \textit{Eucalyptus marginata}) and 12 \textit{P. cinnamomi} isolates.

\textit{Plants.} One-year-old \textit{E. marginata} and \textit{B. hookeriana} seedlings were potted into free draining, 100 mm diameter pots 1 month before spray application. The potting mix contained equal volumes of peat (Floraturf 500, Oldenburg, Germany) and coarse yellow sand. The mix was steam pasteurised at 60°C for one hour and every 60 L of mix was supplemented with the following fertilizers; isobutylidene diurea (31% nitrogen) 50.98 g, KNO\textsubscript{3} 26.33 g, Ca(H\textsubscript{2}PO\textsubscript{4})\textsubscript{2}.H\textsubscript{2}O (Aerophos) 23.53 g, FeSO\textsubscript{4} 17.25 g, FeO 35.29 g, dolomite 47.06 g, gypsum 31.37 g, trace elements 6.27 g. Plants were fertilised with approximately 4 g per pot of Osmocote plus (Scotts Europe BV, Heerlen, Netherlands) 2 weeks after they were potted.

\textit{Phosphite application.} Plants were maintained in a tunnelhouse, under 50% shadecloth for one month before they were sprayed with Foli-R-Fos 400 (400 g/L phosphite present as mono-di-potassium phosphite, Unitec Group Pty Ltd). The plant foliage was sprayed to run-off with backpack spray units (Volpi, 15 L) which contained phosphite (0 or 5 g phosphite/L) and 0.25 g/L of the surfactant Synertrol Oil (Organic Crop Protectants, Australia). The soil in each pot was covered with plastic to prevent
phosphite from leaching into the soil. Over the subsequent 2 days the soil was hand watered to prevent the phosphite from being washed off the leaves.

**P. cinnamomi isolates and inoculum production.** *P. cinnamomi* isolates were chosen according to their sensitivity to phosphite *in vitro* (Wilkinson *et al.* in press-b). The isolates were phosphite tolerant: MP32, MP97, MP125 and MP94-17; intermediate: MP94-10, MP94-25, MP94-26, MP94-48; and phosphite sensitive: MP62, MP80, MP127, MP94-03. Growth of the tolerant, intermediate and sensitive isolates *in vitro* were inhibited by 73-77%, 85-87% and 94-100%, respectively, on Ribeiro’s Modified Medium (RMM) containing 0.05 g phosphite/L. The isolates were obtained from dying *Eucalyptus marginata* that had never been exposed to phosphite. To obtain some uniformity between the isolates after long-term storage agar and to ensure that they were still able to infect plant tissue, they were inoculated into separate *E. marginata* seedlings, allowed to develop a lesion and then plated onto a *Phytophthora* selective medium (NARPH) ((Shearer *et al.* 1995) modified with 10 mg/L Rifampicin (Rifadin, Hoescht Marion Ruessel, Italy)).

Miracloth (Calbiochem Corporation) was washed, cut into 6 mm discs and sterilised on 3 consecutive days for 20 min at 121°C. The sterilised discs were placed onto 10% V8 agar plates (Byrt *et al.* 1979) and inoculated by placing, on the centre of the plate, colonised agar cut from the growing edge of a five day old *P. cinnamomi* culture. Plates were incubated in the dark at 24°C for 6 days by which time the culture had completely colonised the discs.

**Plant inoculation.** The plants were moved to a temperature controlled glasshouse 2 weeks before they were inoculated with *P. cinnamomi*. The plants were inoculated 3 months after they were sprayed with phosphite. *B. hookeriana* and *E. marginata* were inoculated on the main stem 5 or 10 cm from the base of the plant, respectively. The plants were inoculated by making an incision through the periderm into the phloem in an upward movement, leaving the top of the cut still attached to the plant. A *P. cinnamomi* colonised Miracloth disc was then placed under the flap, the wound sealed with Parafilm (American National Can™ Chicago IL) and flagging tape tied around the inoculation point. The ambient temperature was recorded in the glasshouse.

**Plant harvest.** The plants sprayed with 0 and 5 g phosphite/L were harvested 7 and 14 days after inoculation, respectively as *P. cinnamomi* girdled the plants treated with 0 g phosphite/L within 7 days of inoculation. The phosphite treated plants, were left for 14 days to allow time for any potential differences between isolates to occur. To determine total colonisation by *P. cinnamomi*, the stem above the inoculation point was cut into 1 cm long sections, halved longitudinally and plated sequentially onto NARPH. The growth rate of each isolate was determined by dividing the total number of stem pieces from which *P. cinnamomi* was recovered by the number of days the plants were inoculated. Three stems of each plant species treated with 0 g phosphite/L and ten stems of plants treated with 5 g phosphite/L were chosen at random and analysed for phosphite and phosphate using high performance ion chromatography (HPIC) (Roos *et al.* 1999).

**Statistical analysis.** The growth rate data were analysed using analysis of covariance with the independent variables block, phosphite spray concentration, plant species and *P. cinnamomi* isolate.
Diameter of the plant stem at the point of inoculation was included as a covariate. Growth rate data were square root transformed to satisfy the assumptions of homoscedasticity and normality for parametric analysis. The coefficient of determination ($r^2$) was used to determine if there was a correlation between (a) growth of the isolates in vitro on RMM containing no phosphite and the growth of the isolates in plants sprayed with 5 g phosphite/L, and (b) inhibition of the isolates on RMM containing 0.05 g phosphite/L and inhibition of the isolates in plants sprayed with 5 g phosphite/L.

The percentage inhibition of each isolate was calculated for each plant species using the following formula: (average growth rate in plants not treated with phosphite – growth rate in a plant treated with phosphite)/ average growth rate in plants not treated with phosphite. These data were then analysed using analysis of variance with P. cinnamomi isolate and plant species as the independent variables. All data were analysed using Statistica for Windows (Statsoft, version 5.5 1999).

**Mined area**

The methods used for the trial in the rehabilitated mined area were the same as those used in the glasshouse trial unless stated otherwise.

**Trial design.** The experiment was a split plot design established on an 18-month-old rehabilitated bauxite minepit in E. marginata forest of western Australia (Alcoa World Alumina Australia Jarrahdale mine, approximately 50 km south-east of Perth). There were 3 blocks each containing 3 main plots which were sprayed with 0 (control), 5 or 10 g phosphite/L. Plants were inoculated at one of three times after phosphite application, with one of 5 P. cinnamomi isolates. Within each main plot there were 60 E. marginata seedlings, four of these plants were inoculated with the same isolate of P. cinnamomi at each inoculation time.

**Phosphite application.** In September (spring) plants were sprayed to run-off with 0, 5 or 10 g phosphite/L (Foli-R-Fos 400) and Synertrol Oil (0.25 g/L) using backpack spray units. The soil was not covered in the minesite trial.

**P. cinnamomi isolates and plant inoculation.** Five of the isolates that had been used in the glasshouse trial, and which had ranged in sensitivity to phosphite in vitro (Wilkinson et al. in press-b), were used; two tolerant (MP97, MP125), two sensitive (MP62, MP94-03) and one intermediate isolate (MP94-48). Plants were inoculated 2 (spring), 35 (autumn) and 52 (spring) weeks after phosphite application. The autumn inoculation was 14 days after the first major rainfall event after summer. Earlier inoculation was not done as low plant water potential has been shown to affect the ability of P. cinnamomi to colonise E. marginata (Bunny et al. 1995). On each plant, a side stem 0.7-4.3 cm in diameter was inoculated at least 15 cm above the lignotuber.

**Plant harvest.** The plant stems were harvested 27, 37 and 36 days after inoculation for the 2, 35 and 52 week inoculation times, respectively. At each inoculation time extra plants which had not been treated with phosphite, were inoculated and harvested 7-14 days later to determine the approximate growth rate of P. cinnamomi. This growth rate was then used to estimate the appropriate time to harvest the experimental plants which was when the average growth in the controls was at least 3 cm. Five stems of
similar size to the inoculated stem were chosen at random from each phosphite treatment for analysis of phosphite and phosphate.

To determine whether phosphite had an effect on plant growth, stem diameters (5 cm above soil level) and heights were measured before the plants were sprayed and again prior to inoculation.

**Statistical analysis.** The *P. cinnamomi* growth rate data were analysed using analysis of covariance with the independent variables *P. cinnamomi* isolate, the phosphite spray concentration, inoculation time and block. Stem diameter at the point of inoculation was included as a covariate. The growth rate data were square root transformed to satisfy the assumptions of homoscedasticity and normality for parametric analysis.

The percentage growth inhibition was calculated and analysed using the methods described for the glasshouse trial. Fisher’s least significant difference (LSD) test ($\alpha=0.05$) was used to determine the difference in the inhibition of the isolates at each inoculation time.

Height and diameter increments were calculated for each inoculation time and analysed by ANOVA.

**RESULTS**

**Glasshouse.** The maximum and minimum temperatures were 32.3°C and 14.4°C, respectively, on the day the plants were sprayed. Over the period the plants were infected the average daily maximum and minimum temperatures were 29.6°C (SE ± 1.3°C) and 23.1°C (SE ± 0.5°C), respectively.

The growth rate of *P. cinnamomi* was reduced in plants sprayed with 5 g phosphite/L when compared with those sprayed with 0 g phosphite/L ($p<0.001$). There was also a significant interaction between plant species and applied phosphite concentration ($p<0.001$). Isolates grew faster in *E. marginata* than *B. hookeriana*, in plants not sprayed with phosphite whilst in plants sprayed with 5 g phosphite/L, isolates grew faster in *B. hookeriana* than *E. marginata* (data not shown).

There was a difference in the inhibition of the isolates in the two plant species ($p<0.001$). The growth of the isolates was less inhibited in *B. hookeriana*, sprayed with 5 g phosphite/L, than in *E. marginata* sprayed with the same level of phosphite (Fig. 3.1). There was no significant (*B. hookeriana* $p=0.22$, *E. marginata* $p=0.27$) difference between the 12 *P. cinnamomi* isolates when each plant species was analysed separately.
Fig. 3.3. Inhibition (%) of the growth (± SE) of twelve Phytophthora cinnamomi isolates that were inoculated into (A) Banksia hookeriana and (B) Eucalyptus marginata seedlings treated with 5 g phosphite/L in a glasshouse. The in vitro response is shown below the isolate code numbers.

There was little or no correlation (B. hookeriana $r^2=0.26$ p=0.09, E. marginata $r^2=0.04$ p=0.52) in the growth of the isolates in vitro on agar medium (RMM) without phosphite and growth of the isolates in plants sprayed with 5 g phosphite/L. There was also no significant correlation (B. hookeriana $r^2=0.14$ p=0.24, E. marginata $r^2=0.03$ p=0.62) in the inhibition of the isolates on agar containing 0.05 g phosphite/L and the inhibition of the same isolates in plants sprayed with 5 g phosphite/L (data not shown).

B. hookeriana stems had higher concentrations of phosphite and phosphate than E. marginata stems (Table 3.1).

Table 3.1. Mean phosphite and phosphate concentrations in the stems of glasshouse grown plants which had been sprayed with phosphite 3 months previously.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Phosphite applied (g/L)</th>
<th>In planta concentration (mg/g dry wt ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate</td>
<td>Phosphate</td>
</tr>
<tr>
<td>Banksia hookeriana</td>
<td>0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.0 ± 0.3</td>
</tr>
</tbody>
</table>

| Eucalyptus marginata  | 0                       | 0 ± 0                                     |
|                       | 5                       | 0.7 ± 0.1                                 |

$^a$0 g phosphite/L n=3, 5 g phosphite/L n=10
**Mined area.** The average daily minimum temperatures on the minesite during the period the plants were inoculated were similar for the three inoculations, whilst the average daily maximum temperatures varied from 17-20.3 °C (Table 3.2).

Table 3.2. The minimum and maximum temperatures (°C) and rainfall (mm) during the periods inoculated plants were present on the minesite.

<table>
<thead>
<tr>
<th>Time after spray (weeks)</th>
<th>Temperature (°C)</th>
<th>Rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average minimum</td>
</tr>
<tr>
<td>2</td>
<td>3.1 - 26.6</td>
<td>7.8</td>
</tr>
<tr>
<td>35</td>
<td>1.4 - 20.1</td>
<td>8.1</td>
</tr>
<tr>
<td>52</td>
<td>4.1 - 28</td>
<td>8.7</td>
</tr>
</tbody>
</table>

*a* plants were inoculated with *P. cinnamomi* after treatment with phosphite.

*b* rainfall while the plants were inoculated.

The *P. cinnamomi* growth rate in *E. marginata* stems were similar to inhibition results so only the latter are presented (Fig. 3.2). There was a highly significant (*p*<0.001) difference in the inhibition (%) between the isolates at the three inoculation times and the inoculation times were analysed separately to determine if there was a difference in the inhibition of growth of the isolates at each time. These analyses showed that there was a difference in the inhibition of the isolates and Fisher’s LSD post hoc test showed that, in the first inoculation, isolate MP125 was less inhibited (*p*<0.05) than the other four isolates in plants treated with phosphite (Table 3.3). However, in the 35 week inoculation time, isolate MP97 was less inhibited (*p*<0.05) than the other four isolates. Finally, in the third inoculation (52 weeks after phosphite application), isolate MP94-03 was less inhibited (*p*<0.05) than all other isolates except MP94-48 (Fig. 3.2, Table 3.3).

There was a significant (*p*=0.01) reduction in the growth rate of all of the *P. cinnamomi* isolates in phosphite treated plants compared with the non-phosphite treated plants when they were inoculated 2 weeks after phosphite was applied. However, from the inoculation 35 or 52 weeks after spray application there was no significant (35 weeks *p*=0.22, 52 weeks *p*=0.44) difference in the growth rates of the isolates in plants treated or not treated with phosphite.
Fig. 3.4. Inhibition (%) of the growth (± SE) of five Phytophthora cinnamomi isolates inoculated into Eucalyptus marginata seedlings in a rehabilitated minepit (A) 2 weeks, (B) 35 weeks or (C) 52 weeks after spray application of 5 (□) or 10 (■) g phosphite/L.
Table 3.3. The average percent inhibition of five *Phytophthora cinnamomi* isolates inoculated into *Eucalyptus marginata* seedlings which had been sprayed with 5 or 10 g phosphite/L. Percent inhibition has been averaged for the combined phosphite concentrations.

<table>
<thead>
<tr>
<th>Time after spray (weeks)</th>
<th>Isolate</th>
<th>Inhibitiona (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>MP62</td>
<td>79 b</td>
</tr>
<tr>
<td></td>
<td>MP94-03</td>
<td>81 b</td>
</tr>
<tr>
<td></td>
<td>MP97</td>
<td>86 b</td>
</tr>
<tr>
<td></td>
<td>MP125</td>
<td>59 a</td>
</tr>
<tr>
<td></td>
<td>MP94-48</td>
<td>83 b</td>
</tr>
<tr>
<td>35</td>
<td>MP62</td>
<td>6 b</td>
</tr>
<tr>
<td></td>
<td>MP94-03</td>
<td>10 b</td>
</tr>
<tr>
<td></td>
<td>MP97</td>
<td>22 a</td>
</tr>
<tr>
<td></td>
<td>MP125</td>
<td>22 b</td>
</tr>
<tr>
<td></td>
<td>MP94-48</td>
<td>27 b</td>
</tr>
<tr>
<td>52</td>
<td>MP62</td>
<td>18 b</td>
</tr>
<tr>
<td></td>
<td>MP94-03</td>
<td>29 a</td>
</tr>
<tr>
<td></td>
<td>MP97</td>
<td>9 b</td>
</tr>
<tr>
<td></td>
<td>MP125</td>
<td>16 b</td>
</tr>
<tr>
<td></td>
<td>MP94-48</td>
<td>0 ab</td>
</tr>
</tbody>
</table>

aThe letters indicate a significantly (α=0.05) different group within each time after spray.

The phosphite concentration in the stems was highest in the plants sprayed with 10 g phosphite/L and harvested 6 weeks after phosphite application (Table 3.4). Forty-one weeks after phosphite application there was an average 6.4 and 8 fold decrease in the phosphite concentration in the stem of plants treated with 5 and 10 g phosphite/L, respectively. There was no detectable phosphite in the stems 58 weeks after phosphite was applied (Table 3.4). The phosphite concentrations were 8.4 times higher in the stems of glasshouse *E. marginata* 3 months after spray application than in minesite plants sampled 6 weeks after spray application (Tables 3.1 and 4.4).

Application of phosphite had no significant effect on the height or diameter growth of the plants at either 35 (height p=0.57, diameter p=0.42) or 52 (height p=0.69, diameter p=0.41) weeks after treatment (data not shown).


Table 3.4. Phosphite and phosphate concentrations in the stems of *Eucalyptus marginata* seedlings sprayed with 0, 5 or 10 g phosphite/L (n=5)

<table>
<thead>
<tr>
<th>Time after spray (weeks)</th>
<th>Phosphite applied (g/L)</th>
<th>Phosphite mg/g dry wt ±SE</th>
<th>Phosphate mg/g dry wt ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0</td>
<td>±0</td>
<td>0.361±0.007</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.083 ±0.016</td>
<td>1.248±0.245</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.104 ±0.011</td>
<td>0.865±0.138</td>
</tr>
<tr>
<td>41</td>
<td>0</td>
<td>±0</td>
<td>0.617±0.213</td>
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<tr>
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<td>0.790±0.189</td>
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<td>0.936±0.232</td>
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<tr>
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<td>0.747±0.150</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>±0</td>
<td>0.862±0.088</td>
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</table>

**DISCUSSION**

In the glasshouse, the foliar application of phosphite decreased the colonisation of 12 *P. cinnamomi* isolates 3 months after application in *B. hookeriana* and *E. marginata* seedlings. However, there was no correlation between the *in vitro* and *in planta* phosphite sensitivity of these isolates. These results are in contrast with those of Dolan and Coffey (1988) and Fenn and Coffey (1989) who found a strong correlation between the *in vitro* and *in planta* sensitivity to phosphite of chemically mutated strains of *P. palmivora*, *P. capsici* and *P. parasitica* var *nicotianae*. However, our results agree with those of Bunny (1997) and Bashan et al. (1990) who found no correlation between the *in vitro* and *in planta* sensitivity of *P. citricola* and *P. infestans* isolates, respectively. An obvious difference between those studies reporting a correlation between *in vitro* and *in planta* sensitivity and those not, is the use of chemically mutated strains of *Phytophthora* compared with ‘naturally’ tolerant isolates. Chemical mutation of an isolate may change more than just the phosphite tolerance of an isolate, it may also change characters such as aggressiveness of the pathogen. The relationship between the genotypic and phenotypic behaviour of the chemically mutated tolerant strains of *Phytophthora* and wild type isolates is not known. The isolates used in our experiments represent the full range of sensitivities of the Western Australian isolates currently tested *in vitro* (Wilkinson et al. in press-b). However, growth of our isolates was more inhibited by phosphite *in vitro* than the most tolerant isolates used by other workers (Dolan et al. 1988; Fenn et al. 1989). The results of *in vitro* sensitivity of an isolate to phosphite will only be of value if the data correlates with that *in planta*.

The phosphite concentrations detected in the stems in the glasshouse trial were 40 and 14 times higher in *B. hookeriana* and *E. marginata* stems, respectively, than the highest levels used *in vitro* (0.05 g/L). However, the growth inhibition of the isolates *in planta* was less than *in vitro*. If phosphite directly inhibits growth *in planta* when it is present at high levels then it would be expected that the 12 *P.*
*cinnamomi* isolates would have been more inhibited *in planta* than *in vitro*, and this was not the case. A confusing factor in this experiment was the high level of phosphate in the stems. In the glasshouse trial, the stem phosphate was considerably higher than the total phosphorus (P) predicted from the literature. *E. marginata* seedlings grown in jarrah forest soil for 27 months with no added fertiliser had 0.17-0.39 mg P/g dry stem weight (Dell *et al.* 1985). Foulds (1993) measured the P concentration in a large range of Western Australian native plant species and found that Proteaceae and Myrtaceae had 0.1-2.2 mg P/g and 0.5-2.5 mg P/g dry shoot weight, respectively. However, in fertilised plants P concentrations may be elevated in stems where excess P is stored as phosphate (Dell *et al.* 1987). Phosphate and phosphite are transported into *Phytophthora* by both a low and high affinity uptake system (Barchietto *et al.* 1989; Griffith *et al.* 1989a; Griffith *et al.* 1989b). The low affinity system operates when the phosphate concentration is high and the high affinity system operates when phosphate is limiting. Both transport systems can translocate phosphite. Griffith *et al.* (1993) found that the growth of phosphite sensitive isolates of *P. palmivora* were inhibited at all phosphate concentrations, whereas tolerant isolates were only inhibited by phosphite when phosphate was limiting. This does not appear to be the case for *P. cinnamomi* as the phosphite tolerant isolates did not grow faster than the sensitive isolates in plants which contained high levels of phosphite and phosphate.

Phosphite was more effective in inhibiting the growth of the *P. cinnamomi* isolates in *E. marginata* than in *B. hookeriana* despite the tissue concentrations of phosphite and phosphate in the stems of *B. hookeriana* being higher than in *E. marginata*. It has been suggested that phosphite may not be as effective in plants with a less dynamic defense system (Smillie *et al.* 1989). This may explain the difference in the effectiveness of phosphite in *B. hookeriana* and *E. marginata*. Alternatively, high levels of phosphate in the *B. hookeriana* stems may have affected the efficacy of phosphite by competing with, and thus decreasing phosphite uptake into *P. cinnamomi*.

Plants grown in pots in a glasshouse had much higher levels of phosphite in their stems than plants in the field. The phosphite concentrations in the stems of *B. hookeriana* and *E. marginata* in the glasshouse trial were high relative to the concentrations observed in *E. marginata* in the minesite trial. In a previous trial, in the same rehabilitated minepit, *E. marginata* seedlings treated with 5 g phosphite/L 3 weeks prior to analysis had 0.06 mg phosphite/g dry stem weight (Wilkinson *et al.* in press-a). *Banksia telmetia*, in the field, which had been misted twice with 40 g phosphite/L (ultra low volume droplets) had 0.11 mg phosphite/g dry leaf weight (Komorek *et al.* 1998). Fairbanks *et al.* (in press) found that *Corymbia calophylla* (grown in pots in a glasshouse), that had been misted with 40 g phosphite/L, had equivalent levels of phosphite in their stems to plants which had been sprayed to run-off with 5 g phosphite/L. The *C. calophylla* which were sprayed to run-off with 5 g phosphite/L contained 0.85 mg phosphite/g dry weight stem 7 days after application (Fairbanks *et al.* in press).

Higher phosphite uptake in glasshouse grown plants may be due to more favourable conditions for uptake of the chemical by the plant. High humidity is thought to accelerate sorption of herbicides and pesticides through the stomata and cuticle by slowing the drying time of the spray droplet (Ruiter *et al.* 1992). Uptake of chemicals through stomata only occurs while the spray deposits remain liquid, with
uptake via the cuticle once the spray has dried (Stevens et al. 1992). Some of the advantages of stomatal uptake are that rapid uptake decreases the time for photodegradation and volatilisation, penetration into the tissue is increased by the thin cuticle lining and the large surface area within the stomata and once the chemical is within the leaf it is in closer proximity to the vascular tissue which may enhance systemic movement (Stevens et al. 1992).

In the minesite trial, *P. cinnamomi* growth was inhibited in phosphite treated plants when they were inoculated 2 weeks after phosphite application. However, there was no inhibition of growth when the plants were inoculated 35 or 52 weeks after phosphite was applied. These results indicate that *E. marginata* growing on rehabilitated minepits would have to be sprayed every 6-12 months to prevent colonisation by *P. cinnamomi*. Komorek et al. (1998) found that a double application of phosphite (ultra-low volume droplet size) 4 weeks apart increased the longevity of protection of two *Banksia* species. It has been shown that phosphite concentrations greater than 10 g phosphite/L, sprayed to run-off, can cause severe phytotoxicity to a range of plant species ((Pilbeam et al. in press) and personal observations). Therefore, to increase the time that the plants are protected from the pathogen, repeated sprays may be of more benefit than increasing the concentration of phosphite applied.

When plants were inoculated 2 weeks after phosphite application in the minesite trial, isolate MP125 was less inhibited than the other isolates. This isolate was also the most tolerant of the 5 isolates to phosphite *in vitro*. However, the other isolate found to be tolerant to phosphite *in vitro* (MP97) was the most inhibited in plants on the minesite.

The difference in results for the glasshouse and minesite trial, with respect to isolate growth inhibition, may have been due to the difference in phosphite concentration in the plant stems. The phosphite concentration in the stems of the glasshouse plants may have been high enough to disrupt the cell wall composition of all of the isolates, whereas in the minesite trial the stem phosphite concentrations were lower and the cell wall composition of isolate MP125 may have been less affected than other isolates. When plants were inoculated 35 or 52 weeks after phosphite application there was no significant difference in the growth rate of the isolates in phosphite treated plants when compared with plants not treated with phosphite. Therefore, any difference in the inhibition of the isolates may have been due to their growth at different times of the year when the temperature and water status of the plants would be different.

In our studies, there was no correlation between the *in planta* and *in vitro* sensitivity of *P. cinnamomi* isolates to phosphite, thus predictions about the *in planta* sensitivity of *P. cinnamomi* may not be reliable if they are made solely from the results of *in vitro* trials. They also show that caution should be taken when extrapolating results from glasshouse to field trials. We have shown that phosphite was effective for less than 35 weeks in *E. marginata* seedlings sprayed to run-off with 5 or 10 g phosphite/L in the field. This finding has management implications in rehabilitated minesites and possibly to native plant communities. To prevent the death of *E. marginata*, the vegetation may need to be treated every 6-12 months or alternative treatments, such as multiple applications of phosphite need to be investigated. If plants need to be treated every 12 months, phosphite may not be a cost effective tool in controlling *P.
cinnamomi in large areas of vegetation. Research into the phosphite uptake process in plants may help to improve uptake and thus, longevity of the fungicide within native plants in the field.

**Recommendations**
- Care should be taken when extrapolating results from *in vitro* to *in planta* and from the glasshouse to the field.
- Phosphite must be reapplied every 6 months to *E. marginata* seedlings growing in a rehabilitated minepit to maintain protection.

**Recommendations for future research**
- Investigate methods such as multiple applications of phosphite or different adjuvants to increase the uptake of phosphite by plants.
- Investigate the mechanisms of phosphite uptake in a range of plant species.
3.3 The Effect of Phosphite on Phytophthora cinnamomi Zoospore Production In Planta

(submitted to Plant Pathology for publication)
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Abstract

The efficacy of phosphite to control the production of zoospores from *Phytophthora cinnamomi* infected trees grown in a glasshouse and in a revegetated mined area was examined. *Banksia grandis* and *Eucalyptus marginata* seedlings in the glasshouse and *E. marginata* seedlings in the minepit were sprayed with 0, 5 and 10 g phosphite/L. In both trials zoospores were produced from infected tissue of plants treated with all concentrations of phosphite. In the glasshouse, spray application of 5 and 10 g phosphite/L significantly reduced the production of zoospores from both *B. grandis* and *E. marginata* seedlings. In the mined area, there was a similar, though non-significant reduction in the number of zoospores produced from phosphite treated and non-treated *E. marginata* seedlings. However, the average number of zoospores produced was greater in plants not treated with phosphite (1.75 zoospores/mL) than from plants treated with 5 or 10 g phosphite/L (0.04 and 0.09 zoospores/mL, respectively). *Pimelea ferruginea* leaves were used to bait the water surrounding the plants in the mined area to determine if the zoospores produced from phosphite treated plants were able to infect plant material. Significantly more baits were infected by zoospores from plants not treated with phosphite compared to plants treated with 5 or 10 g phosphite/L. These results suggest that phosphite reduces, but does not prevent viable zoospore production from plants. Thus, phosphite application may not remove the risk of *P. cinnamomi* spreading from infested, sprayed areas.

Introduction

*Phytophthora cinnamomi* Rands is a major pathogen of native plant communities in the southwest of Western Australia. It affects approximately 14\% of the northern *Eucalyptus marginata* (jarrah) forest (Davison *et al*. 1989) and one quarter of the 9000 plant species native to the southwest of Western Australia may be susceptible (Wills 1993). Until recently, the main method available for control of *P. cinnamomi* in native vegetation was through mapping of diseased areas and the use of hygiene strategies to reduce further spread of the disease. In 1989, it was first reported that phosphite was able to control *P. cinnamomi* colonisation in *Banksia grandis*, a plant species native to Western Australia (Shearer *et al*. 1989).

Phosphite is systemic and is transported in the xylem and phloem (Cohen *et al*. 1986). Thus, it can be applied as a root drench, stem injection or as a foliar spray, and be transported to the root tissue, the
primary site of infection by *P. cinnamomi*. It is proposed to use phosphite in natural plant communities and in rehabilitated minepits, to contain the pathogen and decrease the risk of spread into non-infested forest. The Department of Conservation and Land Management (Western Australia) is currently applying phosphite, from light aircraft, to *Phytophthora* infested areas in the south of western Australia where there are plant species and communities threatened by *P. cinnamomi* (Komorek et al. 1997).

In the jarrah forest, *P. cinnamomi* is spread by the production of zoospores which swim or are transported in surface and subsurface water downslope of infested areas (Shea et al. 1983; Kinal et al. 1993). In rehabilitated bauxite minepits, jarrah is initially infected in the base of the stem associated with ponded water in riplines (Hardy et al. 1996; O’Gara et al. 1997). Riplines are corrugations which are formed when a minepit is ripped with a winged tine, prior to revegetation.

Phosphite has been shown to affect the production of sporangia in a range of *Phytophthora* species *in vitro* (Coffey et al. 1985; Dolan et al. 1988; Greenhalgh et al. 1994). All *Phytophthora* species tested have a lower EC$_{50}$ for sporangia production than for mycelial growth *in vitro*, however, it is not known how these results relate to the fungus *in planta*.

The present study was undertaken to determine whether phosphite can prevent the production of sporangia and release of zoospores from *P. cinnamomi* colonised plant tissue. An initial experiment was conducted in a glasshouse followed by an experiment in a rehabilitated bauxite minepit in the jarrah forest of Western Australia.

**Materials and methods**

**Glasshouse**

**Trial design.** The experiment was a randomised complete block design with 3 levels of phosphite (0, 5 and 10 g phosphite/L) and two plant species (*Eucalyptus marginata* Donn ex Sm. and *Banksia grandis* Willdenow) inoculated with *P. cinnamomi* arranged in 10 complete blocks. In addition, there was one non-inoculated control plant (flooded but not treated with phosphite) of each plant species in each block.

**Plants.** One month before inoculation, 9-month-old *E. marginata* seedlings and 1-year-old *B. grandis* seedlings were potted into 100 mm, free-draining pots which contained a peat: sand: bark: compeat (1:6:6:3 v:v:v:v) potting mix. The potting medium was steam sterilised at 60°C for one hour and each 60 L was supplemented with the following fertilisers; isobutylidene diurea (31% nitrogen) 50.98 g, KNO$_3$ 26.33 g, Ca(H$_2$PO$_4$)$_2$.H$_2$O (Aerophos) 23.53 g, FeSO$_4$ 17.25 g, FeO 35.29 g, dolomite 47.06 g, gypsum 31.37 g and trace elements 6.27 g.

The experiment was conducted in February (summer) 1997. The plants were placed in a temperature controlled glasshouse 2 weeks before inoculation to allow them to acclimatise. All plants were watered daily to field capacity and fertilised twice a week with 75 mL of Maxicrop (Multicrop (Aust.)) in 9 L of water. Ambient temperature was monitored in the glasshouse.

**Inoculum production.** An isolate of *P. cinnamomi* (MP94-17) was used. It is relatively tolerant to phosphite (EC$_{50}$ *in vitro* is 9 µg phosphite/mL) and produces abundant zoospores *in vitro* (unpublished
data). Prior to use, the isolate was inoculated into *E. marginata* seedlings, allowed to develop a 1 cm lesion and then reisolated onto P$_5$ARH, a *Phytophthora* selective medium (19). This ensured that the isolate was capable of infecting plant tissue.

The isolate was transferred from P$_5$ARH onto 10% V8 agar (Byrt *et al.* 1979) and grown for five days. Miracloth (Calbiochem Corporation, USA) was washed thoroughly with deionised water and cut into 6 mm diameter discs. The discs were sterilised, placed onto a V8 agar plate and inoculated by placing colonised agar cut from the growing edge of a five day old *P. cinnamomi* culture on the centre of the plate. The plates containing the Miracloth discs were incubated in the dark at 24°C. After six days the discs were completely colonised with *P. cinnamomi* mycelium and were used to inoculate the plants.

**Inoculation.** Plant stems were inoculated 1 cm above the soil level by making a shallow incision through the periderm to the phloem with a sterile scalpel in a downward movement. A colonised Miracloth disc was placed under the flap of periderm inside the incision (Davison *et al.* 1994), the wound was sealed with Parafilm (American National Can™ Chicago IL) to prevent desiccation and flagging tape was tied around the inoculation point to exclude light.

**Phosphite application.** Two days after inoculation the plants were sprayed with phosphite (Fosject 200) which contained 0.25% of the adjuvant, Synertrol Oil (Organic Crop Protectants). The soil was covered with plastic to prevent spray from drenching the soil and the foliage of each plant was sprayed until run-off. Once sprayed, the pots were hand watered for 48 h to prevent the phosphite from being washed off the leaves.

**Flooding.** Plants were flooded to assess zoospore production and release. Immediately prior to flooding, the Miracloth discs were removed from the plant stem to eliminate the possibility of zoospores being produced from the discs. Plants were individually flooded by placing them into 850 mL plastic containers (diameter 11 cm). The containers were filled to 1 cm above the soil level with deionised water to ensure that the inoculation point was flooded. Plants were flooded for 24 h and then left to drain for 24 h before being re-flooded. All plants were flooded 5, 7, 9, 11 and 13 days after inoculation and were left to drain freely on days 6, 8, 10 and 12. The optimum number of times to flood the plants and the number of days after inoculation in which the largest number of zoospores were produced had been determined in a pilot trial (unpublished data).

**Sampling and quantification of zoospores.** At the end of each 24 h flooding period water from above the soil level was removed using a syringe. The sampled water was gently stirred to evenly distribute the zoospores before 5 × 2 mL aliquots were plated onto separate P$_5$ARH plates. The syringe was sterilised between samples using 2% aqueous sodium hypochlorite (Ajax Chemicals, 12.5% w/v) and was then washed twice in deionised water.

The P$_5$ARH plates were incubated in the dark at 24°C. After 24 h the water was poured off the plates and they were returned to the incubator. The plates were checked daily for germinating *P. cinnamomi* zoospores and final counts were recorded after 5 days.

**Plant harvest.** The plants were harvested 14 days after inoculation. The plant stems were excised above the lignotuber and 2 cm of each stem (the portion that had been flooded) was examined in water.
under a light microscope (40 × magnification) and the number of dehisced and non-dehisced sporangia were counted. If sporangia were not observed the stem was plated onto P5ARH to determine if it contained viable *P. cinnamomi*.

**Statistical analysis.** The data were analysed using analysis of variance (ANOVA). The independent variables were plant species, phosphite concentration and block and the dependent variable was the number of zoospore/mL on the last day of sampling (14 days after inoculation). Using single degree-of-freedom contrasts, planned comparisons of the number of zoospores produced were conducted between (i) phosphite treated plants versus untreated plants; and (ii) between the different levels of phosphite. Data were log-transformed to satisfy the assumptions of homoscedasticity and normality of residuals for analysis using ANOVA.

**Mined area**

**Trial design.** The experiment was established in an 18 month-old rehabilitated bauxite minepit (Alcoa World Alumina Australia Jarrahdale mine, approximately 50 km south-east of Perth) in February 1999. There were three levels of phosphite (0, 5 or 10 g phosphite/L) applied to each of 3 blocks. Each block comprised of 16 *E. marginata* plants; 4 randomly distributed non-inoculated control plants (not sprayed) and 4 plants within each phosphite level.

**Inoculation.** Plant stems were inoculated under the bark with a 15 mm diameter Miracloth disc on the southern side, 5 cm above soil level using the same technique described for the glasshouse experiment. Silver ducting tape was wrapped around the inoculation point to reflect heat and to provide a dark environment for the pathogen. The Miracloth discs were removed 7 days after inoculation. The plants were watered twice (approximately 30 L/plant), 4 days prior and 3 days after inoculation. This was to increase the plant water potential, which has been shown to affect the ability of *P. cinnamomi* to colonise *E. marginata* (Bunny et al., 1995).

**Phosphite application.** Seven days after inoculation the plants were treated with phosphite (Foli-R-Fos 400) and Synertrol Oil (0.25%) sprayed to run-off using 15 L spray packs.

**Inoculum receptacle.** A receptacle was constructed around the collar of the plant using a method similar to O’Gara *et al.* (19). Ten litre plastic buckets were cut along one side and a hole slightly larger than the plant stem was cut centrally in the base of the bucket. A strip of Parafilm was wrapped around the stem approximately 1.5 cm below the inoculation point. Blu Tack (Bostik, Australia) was placed over the Parafilm and pressed firmly. The bucket was then placed around the stem and the Blu Tack. Additional Blu Tack was used to ensure that there was a seal between the bucket and the stem. The cut side in the buckets was sealed with cloth-backed ducting tape. Soil was built up around the plant to support the bucket. Minepit soil (250 mL) free of *P. cinnamomi* was placed in each bucket to stimulate zoospore production and simulate natural conditions. The plants were flooded 14 days after inoculation (7 days after spray application) by filling the buckets with 3 L of deionised water, which was topped up daily for 16 days. This mimics conditions of ponding which are found to occur in minepits (15,19). The ambient temperature and the temperature of the water in the buckets were measured.
Sampling and quantification of zoospores. Seven days after initiation of simulated flooding the water was baited by floating 20 *Pimelea ferruginea* Labill. leaves on the water surface. To ensure that zoospores rather than mycelium growing out from the inoculated plant infected the baits, fly wire (mesh size 0.5 mm) was placed around the plant stem approximately 3.5 cm from the stem and the baits were placed on the water between the fly wire and the bucket wall. This ensured that the baits did not touch the stems and be infected by mycelia contact. After 24 h, the leaves were removed from the buckets, blotted dry and placed onto NARPH, a selective agar medium based on the medium used by Shearer and Dillon (23), modified by the addition of 10 mg Rifampicin (Rifadin, Hoescht Marion Ruessel, Italy). The total number of bait leaves infected from each plant was recorded. Water samples were also analysed for zoospores by using a syringe to withdraw a 20 mL sample at 1 cm depth, 3 cm away from the plant stem. The syringe had been acid washed in 2M HCl for 12 h and rinsed thoroughly with deionised water. Three 20 mL aliquots were then placed on separate P5ARH plates which were incubated using the method described previously. This procedure of baiting and sampling the water was conducted every second day (five samples in total) until plants were harvested.

Plant harvest. Thirty days after phosphite application, the plant stems were cut into 1 cm segments, (from the point where the bottom of the bucket was attached up to the top of the waterline), these segments were cut longitudinally and placed onto NARPH to determine stem colonisation. Four stems from each phosphite concentration were harvested for phosphite and phosphate analysis using high performance ion chromatography (Roos *et al.* 1999).

Statistical analysis. The data were analysed using analysis of covariance with independent variables phosphite concentration and block, and the dependent variables either the number of zoospores detected (log-transformed) or the percentage of baits infected (angular transformed) on the last day of sampling. The planned comparisons were described previously. Length of colonisation of the plant stem by *P. cinnamomi* was included as a covariate.

Results

Glasshouse

Temperature. The glasshouse reached a maximum temperature of 39°C and a minimum temperature of 24.8°C. The average daily maximum temperature was 33.8°C (SE ±0.8°C) and the average daily minimum temperature was 26.8°C (SE ±0.3°C). There were three days when the maximum temperature was above 35°C (8, 9 and 12 days after inoculation).

The effect of phosphite on zoospore production. There was no significant difference (p=0.23) in the number of zoospores produced in the water surrounding *B. grandis* and *E. marginata* therefore, results for the two plant species were combined. Phosphite at 5 or 10 g/L significantly (p=0.02) reduced the number of zoospores produced from the flooded *B. grandis* and *E. marginata* seedlings (Fig. 3.5) and there was no significant difference (p=0.28) between the 5 and 10 g phosphite/L treatments. No zoospores were produced from non-inoculated control plants.
Fig. 3.5. The effect of flooding and time after inoculation on the number of Phytophthora cinnamomi zoospores produced in the water used to flood (a) Banksia grandis and (b) Eucalyptus marginata seedlings that had been inoculated with P. cinnamomi and sprayed with 0 (†), 5 (‡) and 10 (†) g phosphite/L (n=10, mean, bars represent the standard error of the mean).

Presence of sporangia on plant stems. Phosphite reduced both the number of sporangia produced from stems of B. grandis and E. marginata seedlings and the number that had dehisced (Table 3.5). Sporangia were not observed on all of the plants from which zoospores were detected, however P. cinnamomi was isolated from all plants that had been inoculated, irrespective of treatment with phosphite.

Table 3.5. Numbers of sporangia and the percentage of dehisced sporangia observed on the stems of glasshouse grown Eucalyptus marginata and Banksia grandis seedlings which had been inoculated with Phytophthora cinnamomi, sprayed with phosphite and flooded and sampled after 14 days. The number of plants from which zoospores and sporangia were detected are also shown (n=10).

<table>
<thead>
<tr>
<th>Host</th>
<th>Treatment (g phosphite/L)</th>
<th>Sporangia / 2 cm of stem</th>
<th>Total number</th>
<th>Dehisced (%)</th>
<th>Sporangia</th>
<th>Zoospores</th>
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<tr>
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<td>61</td>
<td>70</td>
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<td>50</td>
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<td>8</td>
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<tr>
<td></td>
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<td></td>
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<td>30</td>
<td>3</td>
<td>6</td>
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<td>10</td>
<td>20</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Mined area

**Temperature.** The ambient temperature reached a maximum of 44°C for three consecutive days (10, 11 and 12 days after the plants were inoculated) and a minimum of 7°C at 23 days after inoculation. The mean daily maximum and minimum ambient temperature after the plants were flooded was 33.5°C (SE ±1°C) and 14.7°C (SE ±1°C), respectively. The temperature of the water in the buckets did not differ greatly from the ambient temperature with a mean daily maximum and minimum of 31.7°C (SE ±0.7°C) and 16.1°C (SE ±0.8°C), respectively. There was one day when the temperature of the water rose above 35°C (25 days after inoculation).

**The effect of phosphite on zoospore production.** There was a significant (p=0.01) reduction in the percentage of baits infected in the water surrounding plants which had been sprayed with phosphite (5 and 10 g phosphite/L) when compared with plants not treated with phosphite (Fig. 3.6). There was a non-significant (p=0.07) reduction in the number of zoospores produced from plants sprayed with phosphite when compared with plants not treated with phosphite (Fig. 3.6). The range in the number of zoospores produced from plants not treated with phosphite was large (0-40 zoospores/mL). No zoospores were detected in plants not inoculated with *P. cinnamomi*. The length of stem colonised did not correlate with the number of zoospores produced (p=0.3) or baits infected (p=0.6) after adjusting for the treatment (block and phosphite concentration) effects.

![Graph A](image1.png)

**Fig. 3.6.** The effect of treating *Eucalyptus marginata* with 0, 5 and 10 g phosphite/L on (a) the percent of *Pimelea ferruginea* baits infected, (b) the number of zoospores produced and (c) colonisation of the plant stem (n=12, mean, bars represent the standard error of the mean).
**HPIC analysis.** The average phosphite concentration was greater in the stems of plants sprayed with 10 g/L than plants sprayed with 5 g phosphite/L and there was no detectable phosphate in plants not treated with phosphite (Table 3.6). The phosphite concentration in the plant stems ranged from 47-120 and 74-142 µg/g dry wt in plants sprayed with 5 and 10 g phosphite/L, respectively. The phosphate concentration was more variable with 385-2025, 222-923 and 379-505 µg/g dry wt in the stems of plants sprayed with 0, 5 and 10 g phosphite/L, respectively.

### Table 3.6. Phosphite concentration in the stem of *Eucalyptus marginata* seedlings sprayed with 0, 5 or 10 g phosphite/L (n=4).

<table>
<thead>
<tr>
<th>Phosphite applied (g/L)</th>
<th><em>In planta</em> phosphite (µg/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>5</td>
<td>65 (26)</td>
</tr>
<tr>
<td>10</td>
<td>97 (16)</td>
</tr>
</tbody>
</table>

* Standard error of the mean

**Discussion**

This study is the first to examine the effect of phosphite *in planta* on the production of zoospores by *P. cinnamomi* in a glasshouse or in the field. In both trials, the application of phosphite decreased the production of sporangia and zoospores, however, it did not prevent their production. Infection of baits in the water surrounding the plants in the mined area trial demonstrated that the zoospores produced from plants sprayed with phosphite were viable and could potentially infect new plants. This has important implications when spraying phosphite to control the spread of *P. cinnamomi* from infested to non-infested areas.

Previous research has shown the ability of *Phytophthora* zoospores to infect plant tissue that has been treated with phosphite (Guest 1986; Dolan *et al.* 1988; Guest *et al.* 1989). Guest *et al.* (1989) investigated the ability of *Phytophthora nicotianae* var. *nicotianae* zoospores to infect *in vitro* grown tobacco seedlings which had been treated with 282 µM fosetyl-Al. Plants were infected and subsequent sporangia production was reduced but not eliminated by treatment. These results are similar to ours in that phosphite did not prevent sporangia production but decreased the number formed. In another experiment where *in vitro* grown sterile tobacco plants were placed in 100 µg/mL of fosetyl-Al or 70 µg/mL phosphorous acid, sporangia were only produced on the control plants (Guest 1986). Zoospore release was not reported in either of the above trials.

Phosphite has been shown to affect the release of *P. cinnamomi* zoospores *in vitro* with 2 µg phosphite/mL in the water surrounding the fungus causing a 39% decrease in zoospore release (Coffey *et al.* 1985). Farih *et al.* (1981) found that 10 µg/mL Efosite-Al in water surrounding *Phytophthora*
Parasitica and P. citrophthora caused 90% and 22% inhibition of zoospore release, respectively. In our glasshouse trial, plants sprayed with phosphite also had less sporangia that had released zoospores.

In the mined area, there was no significant difference between treatments when zoospore numbers were analysed because of the large variation in the number of zoospores from the plants not treated with phosphite. Byrt and Grant (1979) reported a 50% standard error of the mean between flasks when sampling for zoospore production in vitro. They suggested that the variation was due to the difficulties in evenly dispersing the zoospores prior to sampling as they congregate at the surface of liquid and a larger component of the variation was due to different conditions within each flask. There was less variation in the number of zoospores produced within a treatment in the glasshouse than the mined area trial. In the glasshouse trial, all of the water above the soil line was sampled and thoroughly stirred, the plants were relatively close together and thus environmental differences were minimised. In the mined area trial, to avoid dislodging mycelium from the plant stems, the water in the buckets was not stirred prior to sampling, there was also a larger distance between the plants and there were differences in shading and position of the plants in the riplines. However, there was a significant difference between the percentage of P. ferruginea leaves infected. This difference was probably because baiting relies on zoospores infecting the baits rather than sampling for a relatively small number of zoospores in a large quantity of water.

Sporangia and zoospores formed despite the average daily maximum temperature during both trials being greater than 30°C. It has been reported that the optimum temperature for zoospore production by P. cinnamomi in vitro was 18-22°C while at 30°C much lower numbers of zoospores were produced (Halsall et al. 1984). No sporangia were produced in vitro when the incubation temperature was 36°C (Nesbitt et al. 1979). Byrt and Grant (1979) found that no zoospores were produced when they incubated P. cinnamomi at 27°C. In the glasshouse trial, the average daily maximum temperature was 33.8°C and in the mined area trial it was 31.7°C. Therefore, more zoospores may have been produced if the experiments were conducted at a time of year when the temperatures were lower.

In the glasshouse and mined area trials, the number of zoospores produced increased at each sampling time. This was probably due to both the build-up of inoculum levels in the soil and an increase in lesion lengths over time. Longer lesions would give more infected tissue from which sporangia could be produced. It is possible that if the experiments had been extended over a longer period the inoculum may have continued to increase in the soil and water and phosphite may not have continued to limit the production of zoospores. It is also important to note that the plants were flooded almost immediately after phosphite application (3 and 7 days in the glasshouse and mined area trials, respectively) when the phosphite concentration would be high within the plant. Therefore, our results are likely to indicate the maximum inhibition of sporangia production from plants treated with phosphite.

The average phosphite concentration in E. marginata stems was 65 and 97 µg/g dry wt in the stems of plants sprayed with 5 and 10 g phosphite/L, respectively. These phosphite concentrations are higher than those reported by Pilbeam et al. (in press) who recorded 4.2 and 17.2 µg phosphite/g dry wt in Adenanthos barbiger (leaves) and Daviesia decurrens (phyllodes) respectively, which had been sprayed
with 5 g phosphite/L. *P. cinnamomi* colonisation was controlled in these plants. However, it is difficult to compare results for different plant species and different plant parts.

In conclusion, this research has shown that phosphite can decrease the production of zoospores by *P. cinnamomi in planta*. However, zoospores were still produced and these zoospores were able to infect plant material. Thus, phosphite may slow but does not prevent the spread of *P. cinnamomi* from infected plants. More work is required to determine if zoospores produced from phosphite treated plants could infect intact plants and if they could infect phosphite treated plants in the field.

**Recommendations**

- Phosphite reduces but does not prevent the production of zoospores therefore, dieback hygiene measures must be maintained in areas that have been treated.

**Recommendations for future research**

- Investigate the ability of zoospores produced from phosphite treated plants to infect intact plants.
- Determine if zoospores can infect phosphite treated plants in the native vegetation.
3.4 The efficacy of phosphite in reducing the infection and colonisation of the roots of a range of Western Australian native plant species by *Phytophthora cinnamomi*

**Abstract**

Treatment of plants with foliar sprays of 0, 5 and 10 g phosphite/L reduced the growth of *P. cinnamomi* in the roots of *Banksia grandis, Dampiera linearis, Loxocarya cinerea, Loxocarya flexuosa* and *Pattersonia occidentalis*. Phosphite also decreased the recovery of *P. cinnamomi* from inoculated roots of all plant species except *B. grandis* where there was at least 90% recovery from inoculated roots, irrespective of treatment with phosphite. In the first experiment, the first flush of roots were inoculated and harvested and the subsequent flush of roots that grew were also inoculated. When the first flush of roots were inoculated *P. cinnamomi* grew 0-1.4 cm in seven days and was isolated from 0-100% of the inoculated roots from phosphite treated and non-treated plants. When the subsequent flush of roots were inoculated *P. cinnamomi* grew at least 8 cm in seven days in many of the plant roots and was recovered from 100% of the inoculated roots, irrespective of the phosphite spray concentration or plant species. The measured phosphite levels in the roots of *P. occidentalis* were very similar for the two inoculation times. The ambient temperature was higher for the second inoculation and may have affected the efficacy of phosphite. Phosphite did not affect colonisation or recovery of *P. cinnamomi* from *Xanthorrhoea preissii* roots.

**Introduction**

Zoospores are thought to be the most important infection propagule of *Phytophthora cinnamomi*. They are produced when conditions such as moisture and temperature are favourable and are able to swim or are passively transported in the water flow to new hosts. *P. cinnamomi* zoospores are able to penetrate many resistant plant species but these plant species, unlike susceptible species, are able to contain the growth of mycelium once it has invaded. It is thought that susceptible plant species treated with phosphite respond to invasion by *P. cinnamomi* in a similar way to resistant plant species (Guest *et al*. 1990). Thus, treatment of plants with phosphite may not affect the ability of *P. cinnamomi* zoospores to infect plant tissue but may affect consequent colonisation.

All of our trials have relied on the use of underbark wound inoculation as a bioassay to test the efficacy and persistence of phosphite in controlling *P. cinnamomi*. The aim of the present study was to determine if phosphite could control infection and subsequent colonisation by *P. cinnamomi* zoospores in the roots of a range of plant species.
Methods

Experimental design  The experiment was a split plot design, conducted in a glasshouse. There were 2 blocks each contained 3 main plots which were sprayed with 0 (control), 5 or 10 g phosphite/L. Each plot was an aeroponics box, which contained 5 plants of each plant species. The experiment was conducted twice, once in July 1998 and once in April 1999. In the first experiment there were 4 species; *Dampiera linearis* R.Br., *Loxocarya flexuosa* (R.Br.) Benth., *Pattersonia occidentalis* R.Br. and *Xanthorrhoea preissii* Endl. In the second experiment there was an extra species, *Banksia grandis*. Also, *Loxocarya cinerea* R.Br. was included, as *L. flexuosa* was unavailable.

Plants  To encourage root growth, plants were potted into Wynelle pots (with the bottom third of the pot removed) and placed in a drained plastic tray which contained 2:1 v/v Peat (Floratorf 500, Germany)/Perlite (Ausperl P500, Australia) potting mix supplemented with the basal nutrients described previously (Chapter 2.2). Plants were fertilised with approximately 12 g/tray of Osmocote plus (Scotts Europe BV, Netherlands). The plants were left to grow in the trays for 2-3 months before they were placed in an aeroponics system. Briefly, all plant roots below the bottom of the Wynelle pot, which had developed over the 2-3 months, were removed and the plants were inserted into holes that had been cut into the lid of a Nally tub. The plant roots and pot (up to soil level) were suspended into the base of the tub. The plants were watered from the bottom using misting nozzles which sprayed for 8 sec every 20 min. Plants were fertilised with Wuxal liquid (1.25ml/L; Hoechst Schering AgrEvo GmbH) twice weekly.

Phosphite application, plant inoculation, harvest and phosphite analysis  Two weeks prior to inoculation Foli-R-Fos 400 (and 0.25% Synertrol Oil) was applied to run-off to the plant leaves using 1L plastic spray bottles. When plant numbers were sufficient the plants were sprayed with 0, 5 or 10 g phosphite/L. When there were smaller numbers of plants only 0 or 5 g phosphite/L was used. Zoospores of *P. cinnamomi* isolate MP94-48 were used to inoculate the plants. Briefly, to encourage vigorous mycelium growth six 1 x 1 cm squares of *P. cinnamomi* colonised V8 agar were incubated for two days at 24°C, in a 9 cm Petri-dish which contained V8 broth. The *P. cinnamomi* colonised agar squares were washed three times in deionised water then flooded with non-sterile soil leachate and the Petri-dish placed on a light box for 24-48 hrs until sporangia had formed. The *P. cinnamomi* was then cold-shocked by placing the Petri-dish in a fridge (at 5°C) for 30 mins, it was then removed and left at room temperature until zoospores were released.

Plants were removed from the Nally tubs and inoculated by applying a 5 µL droplet of zoospore suspension (approximately 11 000 – 15 000 zoospores/mL) to the root tip using a Gilson pipette. Two to three roots per plant were inoculated depending on the number of roots of sufficient length which were available. The plants were replaced in the Nally tubs after 10 mins, which allowed time for the zoospores to encyst. One week after inoculation, roots were harvested. Ten, 1 cm segments of each root were plated onto a *Phytophthora* selective medium (NARPH) to determine total *Phytophthora* colonisation. The
percent of plants from which *P. cinnamomi* was recovered was also recorded. In the first experiment, after roots from the first inoculation had been harvested (experiment 1a), another flush of roots were allowed to develop and were inoculated approximately 1½ months later (experiment 1b). In the second experiment (1999), only the first sets of roots were inoculated.

*P. occidentalis* roots were harvested from five plants, from each phosphite treatment, at each harvest time, and analysed for phosphite using high performance ion chromatography (Roos *et al.* 1999).

**Results**

**Experiment 1** There was reduced growth of *P. cinnamomi* in phosphite treated *Loxocarya flexuosa* and *Pattersonia occidentalis* when compared to the control plants (Table 3.7). However, colonisation was similar in control and phosphite treated *Dampiera linearis* and *Xanthorrhoea preissii*. The recovery of *P. cinnamomi* was greater from the controls than phosphite treated plants for all species except for *X. preissii* where the recovery was low in the controls (20%) and plants treated with 10 g phosphite/L (20%) and higher in plants treated with 5 g phosphite/L (60%). When the second flush of roots was inoculated (1-2 months after the initial inoculation) *P. cinnamomi* grew at least 8 cm in all plant roots, irrespective of phosphite treatment or plant species (data not shown). Also, *P. cinnamomi* was isolated from 100% of the plant roots.

**Experiment 2** Phosphite decreased the growth of *P. cinnamomi* in *Banksia grandis*, *Dampiera linearis* and *Pattersonia occidentalis* (Table 3.7). The growth of *P. cinnamomi* in *Xanthorrhoea preissii* and *Loxocarya cinerea* was similar in phosphite treated and controls. Recovery of *P. cinnamomi* was less from *D. linearis*, *L. cinerea* and *P. occidentalis* treated with phosphite. There was high recovery of *P. cinnamomi* from *B. grandis* treated with 0, 5 or 10 g phosphite/L. There was a low recovery of *P. cinnamomi* from *X. preissii* and no difference in the recovery from phosphite treated or control plants (Table 3.7).

**Root phosphite levels** The phosphite levels in the plant roots were higher in plants treated with 10 g phosphite/L (mean 2598-3790 µg/g dry weight) than in plants treated with 5 g phosphite/L (mean 1907-2599 µg/g dry weight) (Table 3.7). In Experiment 1, the phosphite levels were lower in the second flush of roots than the first (1a compared to 1b). The phosphite levels in the plant roots higher in the second experiment (Table 3.7).

**Temperature** The temperatures were lowest during the first inoculation of the first experiment (Table 3.8). These lower temperatures correspond to slow growth of *P. cinnamomi* (Table 3.7), relative to other inoculation times. The average maximum and minimum and the highest maximum temperatures were highest during the second inoculation of the first experiment (Table 3.8) and these temperatures correspond to fastest growth of *P. cinnamomi*, relative to other inoculation times.
Table 3.7. The effect of foliar applied phosphite on the infection and colonisation by *Phytophthora cinnamomi* zoospores of the roots of a range of native plant species grown aeroponically. Also, the phosphite levels in the roots of phosphite treated *Pattersonia occidentalis*.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Phosphite concentration (g/L)</th>
<th>Experiment 1a</th>
<th></th>
<th>Experiment 1b</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colonisation (cm ± SE)</td>
<td>Recovery (%)</td>
<td>In planta</td>
<td>Colonisation (cm ± SE)</td>
<td>Recovery (%)</td>
<td>In planta</td>
<td>Colonisation (µg/g dry wt)</td>
</tr>
<tr>
<td>Banksia grandis</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>6.7 ± 0.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>1.2 ± 0.3</td>
<td>90</td>
</tr>
<tr>
<td>Dampiera linearis</td>
<td>0</td>
<td>1.3 ± 0.4</td>
<td>90</td>
<td>100</td>
<td>-</td>
<td>4.8 ± 1.1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.3 ± 0.5</td>
<td>50</td>
<td>100</td>
<td>-</td>
<td>0.7 ± 0.3</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>2.3 ± 0.7</td>
<td>90</td>
</tr>
<tr>
<td>Loxocarya cinerea</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Loxocarya flexuosa</td>
<td>0</td>
<td>1.4 ± 0.3</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>2.8 ± 0.7</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.4 ± 0.3</td>
<td>20</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pattersonia occidentalis</td>
<td>0</td>
<td>0.9 ± 0.3</td>
<td>60</td>
<td>0.9 ± 0.3</td>
<td>60</td>
<td>0 ± 0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.1 ± 0.1</td>
<td>10</td>
<td>100</td>
<td>1157 ± 150</td>
<td>0.5 ± 0.4</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.0 ± 0.0</td>
<td>20</td>
<td>100</td>
<td>2598 ± 301</td>
<td>0.5 ± 0.2</td>
<td>40</td>
</tr>
<tr>
<td>Xanthorrhoea preissii</td>
<td>0</td>
<td>0.9 ± 0.7</td>
<td>20</td>
<td>100</td>
<td>-</td>
<td>0.5 ± 0.3</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.8 ± 0.3</td>
<td>60</td>
<td>100</td>
<td>-</td>
<td>0.2 ± 0.1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.7 ± 0.5</td>
<td>20</td>
<td>100</td>
<td>-</td>
<td>0.5 ± 0.3</td>
<td>30</td>
</tr>
</tbody>
</table>

*Experiment 1a was the first flush of roots, 1b was the second flush of roots and Experiment 2 was a repeat of Experiment 1a.

- not determined
Table 3.8. Minimum and maximum temperatures for the time between inoculation and harvest.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Average maximum (°C)</th>
<th>Average minimum (°C)</th>
<th>Highest maximum (°C)</th>
<th>Lowest minimum (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>23</td>
<td>16</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>1b</td>
<td>27</td>
<td>18</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>20</td>
<td>30</td>
<td>17</td>
</tr>
</tbody>
</table>

* Experiment 1a was the first flush of roots, 1b was the second flush of roots and Experiment 2 was a repeat of Experiment 1a.

**Discussion**

In general, there was a decrease in the recovery of *P. cinnamomi* in phosphite treated plants of all species. This result does not agree with the results of Merwe *et al.* (1994) or Ali *et al.* (1999). Merwe *et al.* (1994) found that phosphite did not affect the ability of *P. cinnamomi* zoospores to encyst, germinate or form appressorium on avocado roots. Ali *et al.* (1999) reported that foliar application of phosphite decreased the number of deaths of two *Xanthorrhoea* spp., growing in *P. cinnamomi* infested soil, but did not affect the number of plants that were infected with the pathogen.

There was a large difference in the growth rate of *P. cinnamomi* in the control plants for the different inoculation times. For example, in *P. occidentalis* roots, *P. cinnamomi* grew an average of 0.9, >8 and 2.8 cm at each of the inoculation times. The only recorded differences between the inoculation times were the ambient temperatures and the time of the year the plants were inoculated. It is not known if factors such as day length and sunlight hours affect the plant/*P. cinnamomi* interaction. However, it is known that ambient temperature affects the growth rate of *P. cinnamomi*, *in planta* (Shearer *et al.* 1987a). It should be noted that while there was large difference in the growth rate of *P. cinnamomi* in Experiments 1(b) and 2, there were not large differences in temperatures. This may be because ambient temperature rather than the temperature inside the aeroponics boxes were measured. As the boxes were black they may have absorbed heat, especially on sunny days which may have resulted in much higher temperatures in the boxes.

Phosphite had no affect on the growth of *P. cinnamomi* when the second flush of roots in experiment 1 was inoculated. This is despite *in planta* phosphite levels remaining high in the second inoculation of Experiment 1. We do not know why there was a sudden loss of efficacy of phosphite for this inoculation. As was mentioned previously, the only measured difference between the inoculation times was the temperature and the effect of temperature on the efficacy of phosphite is not known. In the field it is unlikely that temperatures will be as high as 27°C in soil water unless there are summer rainfall events immediately followed by high temperatures. However, it would be worthwhile to examine how temperature affects the plant, phosphite, *P. cinnamomi* interactions at the cellular and biochemical level. Such studies would help in our understanding of resistance mechanisms.
The phosphite levels in the roots of *P. occidentalis* did not significantly decrease in the second flush of roots of the first experiment even though high levels of phosphite would have been removed in the first flush of roots. This is circumstantial evidence that high levels of phosphite were stored in the leaves of this species. In avocados, phosphite is transported to actively growing regions of the plant (Whiley *et al*. 1995). This would explain the high levels of phosphite in the new roots of *P. occidentalis*.

Phosphite did not affect colonisation or recovery of *P. cinnamomi* from *X. preissii* roots. Pilbeam *et al*. (in press) also found no difference in the colonisation of phosphite treated *X. preissii* when their roots were inoculated with *P. cinnamomi*. They also found that when *X. preissii* were sprayed in autumn, phosphite was not detected in their roots 23 days later. These results indicate that phosphite may not be effective in protecting *X. preissii* against colonisation by *P. cinnamomi*. However, in the current experiment there was low recovery of *P. cinnamomi* from roots irrespective of the phosphite concentration applied. Further work is required to determine if phosphite is effective in *X. preissii*.

Phosphite was effective in reducing the growth of *P. cinnamomi* in 5 of the 6 plant species tested. In a similar experiment, it was found that injection of avocados with phosphite also decreased the colonisation by *P. cinnamomi* zoospores compared to non-treated plants (Merwe *et al*. 1994). These results are only an indication of what may occur in the field as conditions such as temperature, plant nutrient status and water availability may affect the efficacy of phosphite and these factors are very different in the aeroponics boxes. However, our results show that in general phosphite decreased the ability of *P. cinnamomi* zoospores to infect plant roots and the subsequent growth rate of the pathogen. More work is required to determine the efficacy of phosphite in *X. preissii* and the efficacy of phosphite at different temperatures.

**Recommendations for future research**

- To determine the effect of temperature on the efficacy of phosphite in preventing growth of *P. cinnamomi*.
- To determine if phosphite is effective in reducing *P. cinnamomi* colonisation in *Xanthorrhoea preissii*.
Chapter 4

Possible deleterious effects of phosphite

In the evaluation of a fungicide to be used in native plant communities to control the spread of _P. cinnamomi_ it is important to balance any phytotoxic effects with the effectiveness of the control provided. Phosphite has been shown to cause foliar toxicity in a range of horticultural and ornamental plant species and in native plant species.

Phosphite induced phytotoxicity may affect plant reproduction at any point in the reproductive cycle from pollen germination, flowering, fruiting, seed set and germination. The preferred timing of phosphite application is in spring and autumn.

The following studies were initiated to determine:

1. the effects of different phosphite concentrations on phytotoxicity in a range of native plant species (Chapter 4.1), and
2. the effects of phosphite on seed production and viability (Chapter 4.2).

See also Chapters 6.1 and 6.2 for associated studies by PhD students who have examined the effects of phosphite on pollen viability, seed production and viability and the effect of phosphite on beneficial fungi associated with roots of native plants.
4.1 The effect of phosphite concentration on phytotoxicity in a range of plant species at Tiwest

Abstract

The degree of phytotoxicity caused by foliar application of phosphite varied between the phosphite concentration applied and the plant species treated. Application of 5 g phosphite/L caused less than 15% necrosis in 13 out of 18 plant species tested. Higher rates of phosphite affected more plant species to a greater extent with 20 g phosphite/L causing more than 30% foliage necrosis to 12 of the 17 plant species tested. In 8 of these species more than 50% of the foliage was affected. The plant species that were the most affected by all rates of phosphite were *Calytrix flavescens*, *Eremaea asterocarpa* and *Melaleuca scabra*. The symptoms of necrosis were more severe 8½ months than 2 weeks after phosphite was applied. Two years and 7 months after phosphite application, there were very few gross symptoms of phytotoxicity remaining.

Introduction

Phosphite causes phytotoxicity to a wide range of plants including horticultural species (Walker 1989; Anderson *et al*. 1990; deBoer *et al*. 1990; Wicks *et al*. 1990; Seymour *et al*. 1994) and Australian native species (Ali *et al*. 1998; Aberton *et al*. 1999; Barrett 1999; Pilbeam *et al*. in press). At recommended rates symptoms are generally mild but may include leaf necrosis, defoliation and growth abnormalities. In some species the effects are more severe and plants may be killed (Chapter 2.1). Phosphite may induce phytotoxicity as a result of general osmotic stress caused by high concentrations of soluble salts or due to a specific reaction to phosphite ions (Walker 1989). Native plant species vary considerably in their relative sensitivity to phosphite (Barrett, pers com) while individuals within a species may demonstrate varying degrees of phytotoxicity. Phytotoxicity symptoms may be directly related to phosphite uptake and to phosphite concentrations in planta or alternatively species may differ in their tolerance to phosphite. In addition, *in planta* concentrations may vary within the canopy as droplets may be unevenly distributed and translocation throughout the plant may not be uniform (Whiley *et al*. 1995).

There are at least 9000 plant species in southwestern Australia (Wills 1993) and 2540 of these species occur in the kwongan (sandplain) (Lamont *et al*. 1982). If phosphite is to be used widely in the native vegetation it is important to determine the range and severity of phytotoxicity symptoms which may occur and manage the timing and concentration of phosphite applications to minimise these effects.

The aim of this study was to determine the gross phytotoxic effects of varying phosphite concentrations on a range of plant species from different genera and families.
Methods

**Trial design and plant species**  A split plot design experiment was established at the Tiwest minesite (Cataby) which is in the northern sandplain in the south-west of Western Australia. The main plots were 12 m x 12 m in size and were sprayed with 0 (control), 5, 10 or 20 g phosphite/L. There were 4 replicates of each main plot and within these plots there were a total of 7 plants of each species. There were 18 plant species from a range of families and genera (Table 4.1).

**Phosphite application**  The plant foliage was sprayed to run-off with phosphite (Fosject 200), and 0.25% of the adjuvant Synertrol Oil, in February (summer) 1997 using backpack spray units.

**Phytotoxicity rating**  Plants were monitored 2 weeks (summer), 8½ months (spring) and 2 yrs 7 months (spring) after phosphite had been applied. A rating system was developed which was based on the proportion of leaf area affected by necrosis and the proportion of the whole plant which had this leaf area affected. If a plant had two or more leaf symptoms these were added together to give an overall rating for the plant. The rating systems used were:

<table>
<thead>
<tr>
<th>Leaf area affected (%)</th>
<th>Value used in calculations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 10</td>
<td>5.0</td>
</tr>
<tr>
<td>11 - 25</td>
<td>17.5</td>
</tr>
<tr>
<td>26 - 50</td>
<td>37.5</td>
</tr>
<tr>
<td>51 - 75</td>
<td>62.5</td>
</tr>
<tr>
<td>76 - 100</td>
<td>87.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proportion of plant affected (%)</th>
<th>Value used in calculations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 25</td>
<td>12.5</td>
</tr>
<tr>
<td>26 - 50</td>
<td>37.5</td>
</tr>
<tr>
<td>51 - 75</td>
<td>62.5</td>
</tr>
<tr>
<td>76 - 100</td>
<td>87.5</td>
</tr>
</tbody>
</table>

Control plants were also rated and the average value for the control plants was subtracted from the treated plants to determine the effect of phosphite on the plant. When plants were rated 8 ½ months after phosphite was applied some of the leaves which had been damaged by phosphite had dropped off the plant. This symptom could not be accounted for using the above rating system, therefore the percent of bare stem on the plant was recorded and added to the value for the leaf symptoms. For the final monitoring time, plants were rated as dead or alive, presence of flowers (if applicable) and the presence and source of new growth (from the base, middle or top of the plant stems).
Results

Phytotoxicity symptoms on the leaves ranged from leaf tip and margin necrosis to total leaf necrosis and leaf drop. The severity of phytotoxicty symptoms generally increased as the phosphite spray concentration increased however, there were species specific responses. Tolerant species were less than 25% affected by all phosphite concentrations applied while the most sensitive species were at least 25% affected by 5 g phosphite/L and were up to 70% affected by 20 g phosphite/L. Some species showed a marked increase in phytotoxic symptoms (over 50% of the plant) when 20 g phosphite/L was applied but were relatively unaffected (less than 25%) by 5 or 10 g phosphite/L. Similarly, some species showed phytotoxic symptoms when they were sprayed with 10 or 20 g phosphite/L but showed little or no response when they were sprayed with 5 g phosphite/L. In most plant species, the phytotoxic symptoms increased over time (2 weeks vs 8½ months) at either all phosphite concentrations or at the higher concentrations (Table 4.1).

The application of 5 g phosphite/L caused less than 15% damage in 13 of the species up to 8½ months after spray. However, *Calytrix flavescens*, *Eremaea asterocarpa* and *Melaleuca scabra* were up to 36% damaged by 5 g phosphite/L. The higher rates of phosphite affected more plant species to a greater extent with 10 g phosphite/L causing phytotoxicity symptoms on more than 30% of the plant for 8 of the plant species monitored. The species that were worst affected by 10 g phosphite/L were *E. asterocarpa* (50%) and *Stirlingia latifolia* (56%). Application of 20 g phosphite/L caused greater than 30% damage to 12 of the plant species and damaged more than 50% of the plant in 8 of these species. The species worst affected by application of 20 g phosphite/L were *C. flavescens* (62%), *E. asterocarpa* (69%), *M. scabra* (76%) and *S. latifolia* (67%).

When plants were monitored 2 yrs and 7 months after phosphite was applied, in most species the foliage had recovered from earlier phytotoxicity symptoms (data not shown). The only species where phytotoxicity effects were still observed were (i) *Calytrix flavescens* which showed new shoot growth from the middle of stems in plants treated with 20 g phosphite/L, (ii) *Hibbertia hypericoides* in which the new growth on was chlorotic and there were a large number of basal sprouts from plants that were treated with 10 or 20 g phosphite/L, (iii) *Mesomelaena pseudostygia* in which there were dead stems and more new growth in treated with 10 or 20 g phosphite/L and (iv) *Stirlingia latifolia* and *Conostephium pendulum* in which all new growth in plants treated 10 and 20 g phosphite/L was basal.

Discussion

There was a large variation in the response to phosphite between plant species. The most phytotoxicity symptoms were observed 8½ months after plants were sprayed, and in general, as the phosphite concentration applied increased the level of phytotoxicity also increased. Most species were relatively unaffected by the application of 5 g phosphite/L but 10 or 20 g phosphite/L caused as much as 56 and 76% damage to the plant foliage, respectively. The species most affected by phosphite were *Eremaea*
asterocarpa, Melaleuca scabra and Stirlingia latifolia. When plants were examined 2 yrs and 7 months after phosphite was applied there were very few gross symptoms of phytotoxicity remaining in any of the plants. While this is an encouraging result for the use of phosphite in the sandplain it must be emphasised that only a small proportion of the plant species which grow in this area were examined and only gross symptoms were recorded. This study did not examine the long-term consequences of phosphite on plant fitness. The effect of phosphite on plant fitness may be similar to the effects of fire. For example, severe phytotoxicity may predispose plants to pests, disease and environmental extremes such as drought. It may also adversely affect vigour and fecundity of plants. There was some evidence that application of phosphite may have decreased the number of Bossiaea eriocarpa, Eremaea asterocarpa and Petrophile linearis that flowered, however there were insufficient data to confirm this. Barrett (1999) noted that there was significantly reduced flowering in Calytrix leschenaultia and Baeckea preissiana 1 year after phosphite was applied. Reduced flowering may have been due to tip defoliation which decreased bud set, although in B. preissiana there was an abundance of immature flower buds (Barrett 1999). Treatment of Daviesia decurrens with 20 g phosphite/L significantly decreased flowering and pod set, however 5 g phosphite/L had no effect (Pilbeam et al. in press). Two studies in this report found that 5 or 10 g phosphite/L did not affect the height increment of a range of plant species growing in a glasshouse (Chapter 2.2) or Eucalyptus marginata growing on a rehabilitated minepit (Chapter 3.2). Barrett (1999) also found that phosphite did not affect the height increment of Kunzea montana growing in their natural habitat. These results indicate that phosphite is not affecting the vigour of some plant species.
Table 4.1 The average percent of plant affected by foliar application of phosphite at Tiwest (Cataby). Plants were rated 2 weeks and 8½ months after phosphite was applied (n=7)

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Average damage (% ± SE)</th>
<th>Phosphate concentration (g/L)</th>
<th>2 weeks(^{ab})</th>
<th>8½ months(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteaceae</td>
<td><em>Adenanthos cygnorum</em> Diels subsp <em>cygnorum</em></td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>8 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Casuarinaceae</td>
<td><em>Allocasuarina humilis</em> (Otto &amp; F.Dietr.) L.A.S.Johnson</td>
<td>13 ± 6</td>
<td>10± 2</td>
<td>51 ± 6</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Banksia attenuata</em> R.BR.</td>
<td>5 ± 2</td>
<td>3 ± 2</td>
<td>5 ± 2</td>
<td>1 ± 2</td>
</tr>
<tr>
<td>Papilionaceae</td>
<td><em>Bossiaea eriocarpa</em> Benth.</td>
<td>9 ± 4</td>
<td>34 ± 7</td>
<td>44 ± 10</td>
<td>7 ± 5</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Calytrix flavescens</em> A. Cunn.</td>
<td>18 ± 7</td>
<td>38 ± 9</td>
<td>50 ± 9</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Conostephus pendulum</em> Benth.</td>
<td>-3 ± 2</td>
<td>-1 ± 2</td>
<td>21 ± 7</td>
<td>3 ± 6</td>
</tr>
<tr>
<td>Dasygonaceae</td>
<td><em>Dasypogon obliquifolius</em> Nees</td>
<td>7 ± 3</td>
<td>40 ± 6</td>
<td>19 ± 9</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Eremaea asterocarpa</em> Hntiuk</td>
<td>31 ± 7</td>
<td>49 ± 8</td>
<td>71 ± 5</td>
<td>36 ± 7</td>
</tr>
<tr>
<td>Dilleniaceae</td>
<td><em>Hibbertia hypericoides</em> (D.C.) Benth.</td>
<td>5 ± 8</td>
<td>7 ± 3</td>
<td>38 ± 4</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>Papilionaceae</td>
<td><em>Jacksonia floribunda</em> Endl.</td>
<td>2 ± 2</td>
<td>15 ± 8</td>
<td>28 ± 10</td>
<td>15 ± 11</td>
</tr>
<tr>
<td>Dasygonaceae</td>
<td><em>Lomandra hermaphrodita</em> (C.R.P.Andrews) C.A.Gardner</td>
<td>24 ± 9</td>
<td>51 ± 4</td>
<td>53 ± 7</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Restionaceae</td>
<td><em>Loxocarya sp.</em></td>
<td>1 ± 1</td>
<td>10 ± 4</td>
<td>33 ± 9</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Melaleuca scabra</em> R.Br.</td>
<td>25 ± 4</td>
<td>38 ± 5</td>
<td>54 ± 8</td>
<td>13 ± 7</td>
</tr>
<tr>
<td>Cyperaceae</td>
<td><em>Mesomelaena pseudostygia</em> (Kuek.)K.L.Wilson</td>
<td>4 ± 4</td>
<td>14 ± 6</td>
<td>20 ± 4</td>
<td>-2 ± 1</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Petrophile linearis</em> R.Br.</td>
<td>6 ± 4</td>
<td>10 ± 6</td>
<td>15 ± 6</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Rhamnaceae</td>
<td><em>Stenanthemum humile</em> Benth.</td>
<td>5 ± 2</td>
<td>15 ± 7</td>
<td>ND</td>
<td>-9 ± 0</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Stirlingia latifolia</em> (R.Br.)Steud.</td>
<td>7 ± 3</td>
<td>31 ± 8</td>
<td>64 ± 3</td>
<td>4 ± 1.5</td>
</tr>
</tbody>
</table>

\(^{ab}\)Time after phosphite application  
\(^{c}\)Damage determined by rating the total leaf area affected compared with non-sprayed, control plants.  
\(^{c}\)Damage determined by rating the total leaf area affected and the percent of plant with bare stem compared with non-sprayed, control plants.  
ND not determined due to low plant number
This study indicates that the use of 5 g phosphite/L does not appear to produce adverse or long term affects from phytotoxicity symptoms. However, long-term fitness of any species affected by the recommended rate of phosphite (5 g/L), especially seeders, should be examined further.

Recommendations

- Foliar application of 5 g phosphite/L should be used to minimise phytotoxicity symptoms.

Recommendations for future research

- More work needs to be conducted on the long-term effects of phosphite at different rates on the fitness of plants as affected by pests, disease and adverse environmental conditions such as drought.
- More work needs to be done on the effects of phosphite on flowering and pollen and seed viability.
4.2 The effect of phosphite on seed production and viability
Keith McDougall

Abstract

Foliar application of 5, 10 and 20 g phosphite/L had an adverse affect on the germination of *Trymalium ledifolium* seeds. However, only two plants sprayed with each phosphite concentration were assessed. The use of Tetrazollum to test the viability of *Banksia grandis, Daviesia physodes, Lasiopetalum floribundum, Leucopogon verticillatus* and *T. ledifolium* was assessed as a possible method to determine the affect of phosphite on seed viability. Of these five species tested only *B. grandis* could be reliably tested for viability. However, in this species seed is easy to germinate, which is a simpler method for determining viability. It was concluded that these methods were too time consuming for this project.

Introduction

There has been very little work on the effect of phosphite on plant reproduction. Phosphite (2.5 g/L and above) has been shown to affect pollen germination in three perennial Western Australian native plant species and in one annual plant species (Fairbanks, Chapter 6.1). However, seed germination was only affected in the annual plant species sprayed with 5 g phosphite/L (Fairbanks, Chapter 6.1). Flower production was affected in *Baeckea preissiana* and *Calytrix leschenaultia* 5 months after it was applied (Barrett 1999). One-year later, flower production was still lower in the phosphite treated plants. Pilbeam (in press) found that foliar application of 20 g phosphite/L to *Daviesia decurrens* significantly decreased the number of flowers and seed pods produced while 5 g phosphite/L had no affect. In contrast, phosphite did not affect the number or the germination of seeds produced by three *Banksia* spp. (Komorek et al. 1998).

Seed production tends to be extremely variable, spatially and temporally. Some plants within a population will produce abundant seed whilst others will produce little. This may be related to differences in the age of plants (which is not always obvious in vegetation where there are many resprouting species and fire is frequent) or differences in micro-habitat and plants of some species do not produce seed every year. This makes the determination of effects on seed production very difficult. Many replicates are required in seed production studies and all seed produced by each plant must be collected or adequately estimated. Seed traps (e.g. trays with sticky bottoms) have been widely used for estimating seed production. These can be effective in very dense stands of single species (e.g. crops) but are likely to be inadequate in the northern sandplain and jarrah forest communities. Seed predation may also affect production estimates. Seed traps such as bags over foliage and flowers may affect pollination and photosynthetic capacity.

Some types of plants may be easier to study than others: (a) serotinous species (species that store their seed on the plant until fire destroys the parent), and (b) annuals (where viable seed is produced every
year). The inflorescences of *Banksia* spp. on the northern sandplain would make ideal sampling units that could be sprayed individually before, during or after anthesis. *Banksia* spp. also have the advantage that their seed has a high germination rate so that viability studies could be done. Annual species could be grown in the greenhouse, sprayed and monitored carefully for the affects of spraying. Provided pollination occurs in greenhouse grown plants, annual species would be highly suitable for studies of the effect of phosphite on seed production.

Seed viability is the sum of the seed that will germinate immediately and dormant seed (ones that will germinate after some stimulus). Whilst phosphite might affect seed development, any affect will be impossible to interpret in the absence of data on seed production. A significant change in viability following spraying (either positive or negative) will be of little consequence if there is a major change in seed production.

Seed viability is commonly measured using seed lots (samples) of several hundred seeds. Seeds might be germinated on filter paper (to obtain a measure of seed which will germinate immediately) or tested chemically using a standard Tetrazollum test (to obtain an overall measure of viability).

Many jarrah forest species are known to produce seed of low or zero germination. This may indicate that there are germination stimuli that we still do not understand. In such circumstances, it may be worth doing viability tests instead. However, the Tetrazollum test requires a good knowledge of the morphology of the seed of each species tested. Since little is known about the seeds of native plants, the technique is rarely used outside agricultural crop plants.

Two preliminary experiments were carried out to determine the feasibility of studying the effects of phytotoxicity on seed germination and viability.

**Methods**

**Germination**  Seed was collected at Huntly from two plants of two species (*Daviesia physodes* and *Trymalium ledifolium*) at the four concentrations of spraying (0, 5, 10 and 20 g phosphite/L). The seed was collected by putting a net bag over the plants.

*Daviesia physodes* seed is known to germinate optimally following placement in boiling water for 15 seconds (Bell *et al.* 1993). The seeds of both species were treated in this manner before being placed on moistened filter paper above a thin layer of cotton wool in Petri-dishes. The Petri-dishes were placed in a 12°C refrigerator in darkness and checked frequently for the adequacy of moisture. Germination of many jarrah forest seed has been found to be enhanced in darkness. The filter papers were sprayed with a 1% solution of Fongarid™ about 1 week after the start of the experiment when fungal growth was noticed in some *Daviesia* trays. A sub-sample of 50 seeds of *T. ledifolium* and all *D. physodes* seeds were placed on Petri-dishes.

**Seed viability (Tetrazollum test)**  A small sample of seed of *Banksia grandis, Daviesia physodes, Lasiopetalum floribundum, Leucopogon verticillatus* and *T. ledifolium* was obtained from Alcoa's
Marrinup nursery for preliminary viability testing using Tetrazollum Salt (BDH Laboratory Supplies). Seeds were soaked for 48 hours to facilitate removal of seed coatings as staining with the Tetrazollum may be incomplete and result in incorrect viability interpretation unless the solution has adequate contact with the important seed parts (cotyledons, radicle, hypocotyl etc.). After soaking, the seed, coatings of half the seeds were removed (if possible) and the cotyledons separated. Half the seeds, with seed coats intact, were included in the tests to see if complete removal of the seed coat was necessary for staining. All seeds were then placed in a 1% Tetrazollum chloride solution. After 24 hours they were removed and inspected under a dissecting microscope.

**Results**

**Germination** Abundant seed was obtained from all *Trymalium ledifolium* plants (about 70 - 200 seeds). However, less than 10% of the pods produced by the *Daviesia physodes* were filled. All samples, except one, were of less than 10 seeds. This number would not have been adequate for comparison of % viability between treatments.

Germination of *Trymalium ledifolium* seeds was observed 3 weeks after heat treatment. The experiment was stopped after 37 days when no further germination was observed over a 5 day period. No *Daviesia physodes* seeds germinated.

**Table 4.2** Germination of *Trymalium ledifolium* seeds which had been collected from phosphite treated plants (number of plants=2).

<table>
<thead>
<tr>
<th>Phosphite concentration (g/L)</th>
<th>Germination (mean %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
</tr>
</tbody>
</table>

The results cannot be statistically analysed by treatment since there were only two plants per treatment. However, if germination is considered in terms of + or - phosphite, the difference in the means is significant (P<0.001). That is, the germination of seeds from plants sprayed with phosphite was much less than seeds from control plants. This finding is difficult to interpret without some indication of the effect of phosphite on seed production. However, there is clearly some effect of phosphite on reproduction in *Trymalium ledifolium* (Table 4.2).

**Seed viability** The results of the preliminary Tetrazollum tests are summarised below by species:

*Banksia grandis* Seeds were a good size to work with, seed parts were easily recognized and stained well. To obtain adequate staining the seed must be removed from the seed coat and the cotyledons
separated. The seed coat could only be removed after soaking in water (for a day or so). Also, the seed readily germinates so germination tests alone will probably be adequate for estimating viability.

*Daviesia physodes* The seed coat is easy to remove after a day of soaking in water. However, nothing stained after soaking in 1% Tetrazollum chloride for 72 hours. This is very odd because even dead seeds will normally stain to some extent. It is possible that the Tetrazollum test is simply not suitable for this species.

*Lasiopetalum floribundum* These seeds were very small and extremely difficult to dissect even after soaking in water. It was difficult to remove the seed coat without doing considerable damage to the seed.

*Leucopogon verticillatus* There were four small seeds per fruit in this species. These needed to be removed from the hard fruit prior to any test. This was time consuming. All of the seeds inspected were unfilled. If this was normal, many fruits would have to be collected to obtain a large enough sample to test.

*Trymalium ledifolium* The seed was contained within two structures (a capsule containing three seeds and another coating of some sort). The seeds themselves were very small. The cotyledons could not be separated once all the outer structures were removed. A Tetrazollum test would be difficult.

**Conclusions**

Phosphite significantly affected the germination of *Trymalium ledifolium* seeds. However, it must be reiterated that only two plants treated with each phosphite concentration were assessed. It was decided that this work was too large and not within the scope of this project. Aspects of seed and pollen germination were investigated by Meredith Fairbanks whose PhD is entitled ‘The effect of the fungicide phosphite on sexual reproduction of non-native and native plant species of the *Eucalyptus marginata* forest’, also see Chapter 6.1.

Of the five species tested, only *Banksia grandis* could be reliably tested for viability using a standard Tetrazollum test. The seed of this species is also easy to germinate. A less time labour-intensive method of estimating viability would be to germinate seed on filter paper. It was decided that the Tetrazollum test for seed viability required an in depth knowledge of seed morphology and was thus too time consuming for this project.

**Recommendations for future research**

- Select key perennial and annual species and examine seed production and germination after phosphite treatment in plants treated with phosphite before, during or after anthesis.
- Develop assays other than the use of Tetrazollum to examine seed viability.
Chapter 5

Technical issues and operational spray

A number of technical issues were examined in this project to establish the potential of phosphite as a fungicide treatment of native plant communities. These were to:

1. Establish an accurate and reliable method to chemically determine the levels of phosphite in plant tissues. This was necessary to determine:
   a) the levels of phosphite required in plant tissue to effectively control *P. cinnamomi*,
   b) the differences in tissue phosphite levels after the application of different foliar concentrations of phosphite to a range of plant species,
   c) the long-term persistence of phosphite in plant tissues, and
   d) if phosphite was distributed uniformly throughout plant tissues.

To this end Chapters 5.1.1 and 5.1.2 were conducted to develop an effective High Performance Ion Chromatography analytical method to determine the levels of phosphite and phosphate present in plant tissues.

2. All of the activities conducted in this project were experimental and no consideration had been given to the use of phosphite as an operational procedure suitable for mining companies to adopt. Therefore, in Chapter 5.2 an operational procedure was developed to provide a suitable method for mining companies to adopt for the control of small spot infections of *P. cinnamomi*. Particular emphasis was placed on the number of people required to effectively spray and trunk inject approximately 1000 m² of dense and open jarrah forest.
5.1.1 FACILE HIGH PERFORMANCE ION CHROMATOGRAPHIC ANALYSIS OF PHOSPHITE AND PHOSPHATE IN PLANT SAMPLES

(Published in Communications in Soil Science and Plant Analysis)
Gregory H. P. Roos, Christian Loane, Bernie Dell, Giles E. St. J. Hardy.

ABSTRACT: This contribution describes a simple, reliable, high throughput, and cost efficient method for the determination of phosphite and phosphate concentrations in plant samples from a range of plant families using high performance ion chromatography (HPIC). This protocol is a marked improvement over existing methodologies in terms of the practicalities of sample preparation and reliability of subsequent analysis.

INTRODUCTION

In connection with an ongoing research programme aimed at alleviating widespread death in plant communities attributed to Phytophthora spp., a reliable, relatively simple method was required to monitor the phosphite (phosphonate)\(^1\) (1) and phosphate concentrations in tissues of a number of plant species. The increased use of phosphonic acid based derivatives as systemic fungicides in the control of Oomycete pathogens (2, 3) has prompted the appearance in the literature of a number of analytical methods for their assay. The approaches adopted by earlier workers have involved paper chromatography (PC) (4), gas chromatography (GC) (5-10), combined gas chromatography-mass spectroscopy (GC-MS) (11), and variants based on high performance ion chromatography (HPIC) (8, 11-18).

The primary drawback of GC-based applications is the requirement for elaborate sample preparation due to the need to derivatize samples to obtain suitable volatility. In the case of plant organs with a number of different tissue types, this leads to tedious protocols during which the loss of the compounds of interest must be prevented. Thus, the most attractive methodology, especially where large sample numbers are involved, appeared to be offered by single-column HPIC. A survey and testing of the published HPIC-related methods revealed that the majority of the protocols were not applicable for the analysis of large numbers of plant samples. Indeed, either the appropriate commercial columns were no longer available (eg: 16), the reported methodology was restricted only to standards and could not readily be applied to plant material from the field (eg: 15, 17, 18), or the sample preparation was impractical because of its complexity (eg: 8).

The single procedure that had none of the limitations outlined above was reported by Ryder (14), who had developed an appropriate method that relied on a silica-based column. This protocol,

\(^1\) The terms phosphite and phosphonate are both used in the literature to describe various derivatives (including the anions) of phosphonic acid. Although both are currently accepted in terms of IUPAC nomenclature, this contribution utilises phosphite throughout.
which forms the basis for the current method development, proved to be sufficiently robust and could be efficiently adaptable to plant samples. The relatively low cost of the ion column employed by Ryder (1986) (14) compared with the more recent generation of ion columns added to the attractiveness of the procedure.

This investigation aimed at the development of a robust, cost effective, high sample throughput analytical method for the determination of phosphite and phosphate in a wide range of botanical samples. Further, whilst the majority of reported methods are overly directed toward the detection of sub-ppm concentrations, our procedure was focussed primarily on realistic sample concentrations above the 1ppm concentration.

**MATERIALS AND METHODS**

**Plant material:** 0.5g (dry weight) of finely ground (<0.5mm) plant tissue (root, stem, leaves)\(^2\) was shaken by hand with 5ml of de-ionised water. The mixture was allowed to extract overnight (optimised at 10h) at room temperature. Enough extract to obtain 200\(\mu\)l was filtered through a 0.45\(\mu\)m nylon Acrodisc\(^\text{®}\) (Gelman Sciences).

**Chemicals:** Phosphite and phosphate standards were prepared from phosphorous acid (Sigma-Aldrich, 99%) and potassium dihydrogen orthophosphate (Baker, 99%) respectively. The mobile phase was prepared from succinic acid (20mM) (Sigma-Aldrich, 99+) and the pH adjusted to 3.4 with lithium hydroxide.

**Phosphite and phosphate analysis:** The ion chromatographic system consisted of a Waters 501 HPLC pump equipped with a Waters 712 WISP auto-injector and an Alltech 320 conductivity detector. Chromatographic data was recorded and processed using Waters Millenium Chromatography Software version 2.15.01. Sample injections (50\(\mu\)l) were separated using a Vydac 302IC4.6 (0.46 x 25cm) silica-based non-suppressed ion chromatography column,\(^3\) with the succinic acid mobile phase (flow rate 1ml/min). The mobile phase was pre-filtered through a 0.45\(\mu\)m nylon membrane and degassed with helium. Samples were analysed within 24h of preparation to avoid any microbial growth.

Column regeneration was routinely performed every 24h. This consisted of a sequential treatment with 0.5% nitric acid (300ml), water flush, methanol (100ml), water flush, and final re-equilibration with the succinic acid mobile phase. Although the baseline deterioration was very slow, the relatively low cost of these columns suggested that (especially in the case of large sample numbers) the analytical system should have at least two columns in service at any one time. This allows for regular column regeneration without interruption of analysis. Under this protocol, the original columns will, after several hundred analyses, still perform to original specifications.

\(^2\) No distinction was made between roots, stems and leaves since the emphasis was on method development rather than individual analysis type.

\(^3\) Supplied by The Nest Group, Inc. 45 Valley Rd. Southborough, MA, 01772.
RESULTS AND DISCUSSION

The primary determinants of this HPIC analytical protocol were the pH dependent speciation of phosphite and phosphate, as well as the pH operating tolerance of the available ion chromatography columns. Speciation calculations (19) and independent titrations by standard methods (20) confirmed that pH 3.4 provided the optimal situation where the mono-anions of both phosphite and phosphate coexisted essentially to the exclusion of other species. In addition, this was a pH within the optimal operational range of the Vydac ion column. Although alternative HPIC columns were tested (Alltech Allsep Anion; Waters IC-Pak Anion HR; Hamilton PRP-X100), they were found to be far inferior in separation, stability, and resistance to degradation when exposed to complex botanical extracts.

Table 5.1. Comparison of the extraction efficiency of 500µg/g phosphite dosed plant samples by water and succinic acid.

<table>
<thead>
<tr>
<th></th>
<th>Experimental value (µg/g)</th>
<th>Δ (µg/g)</th>
<th>Δ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic acid</td>
<td>494</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>479</td>
<td>21</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>477</td>
<td>23</td>
<td>4.6</td>
</tr>
<tr>
<td>Water</td>
<td>494</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>479</td>
<td>21</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>542</td>
<td>42</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>478</td>
<td>22</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Extraction of plant materials was initially carried out according to protocols presented in the literature (eg: 16, which uses pH 3.5 succinic acid). Subsequent studies revealed that de-ionised water performed equally well (Table 5.1) as the extraction medium, thus further simplifying sample preparation. The use of de-ionised water also resulted in a reduction in base-line noise in the conductivity detector. A time course of extractions showed that the extraction of phosphite and phosphate was optimal after a convenient 10h. (overnight) period (Table 5.2). The stability of the extracted analyte anions was tested to eliminate the possibility of oxidative phosphite to phosphate conversion under the conditions of extraction and analysis. This was shown not to occur in either water or succinic acid medium (Table 5.3). Under the above protocol, the trace for phosphite and phosphate remained well defined, with a stable baseline over a wide range of concentrations.
Table 5.2. The effect of time on the efficiency of extraction of phosphite and phosphate.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Phosphite (µg/g)</th>
<th>Phosphate (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>497</td>
<td>394</td>
</tr>
<tr>
<td>4</td>
<td>521</td>
<td>456</td>
</tr>
<tr>
<td>10</td>
<td>566</td>
<td>477</td>
</tr>
</tbody>
</table>

Table 5.3. Analysis of the stability of the phosphite/phosphate ratio during water and succinic acid extractions.

<table>
<thead>
<tr>
<th>Phosphite spike (µg/g)</th>
<th>Initial phosphate (µg/g)</th>
<th>Final phosphate (µg/g)</th>
<th>Δ (µg/g)</th>
<th>% Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>528</td>
<td>525</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>30</td>
<td>528</td>
<td>535</td>
<td>7</td>
<td>1.3</td>
</tr>
<tr>
<td>40</td>
<td>462</td>
<td>466</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>50</td>
<td>528</td>
<td>533</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>Succinic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>412</td>
<td>415</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>20</td>
<td>410</td>
<td>417</td>
<td>7</td>
<td>1.7</td>
</tr>
<tr>
<td>30</td>
<td>426</td>
<td>425</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>40</td>
<td>428</td>
<td>424</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>50</td>
<td>421</td>
<td>426</td>
<td>5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

To date, after >2000 analyses of standards and a range of plant extracts,4 the Vydac ion columns were found to regenerate cleanly and thus no estimate of overall column life expectancy is available. Although individual chromatograms are run for 40 min to ensure complete elution and column re-equilibration, automation of the HPIC system (via the auto-injector system) allows for up to 30 samples to be routinely analysed daily and the analytical results of the field work that employs this methodology will be reported elsewhere. This method allows reliable analysis of plant material that contains phosphite and phosphate concentrations down to 3-5 ppm. To obtain meaningful analyses below these concentrations, significantly increased sample pre-purification must be carried out.

CONCLUSION

The methodology reported above represents a convenient and inexpensive protocol for the rapid analysis of the phosphite and phosphate concentrations in plant samples. With the expanding interest in diverse field trials of phosphonic acid based derivatives, the simplicity of the above methodology should be appreciated.

4 No attention was paid to plant diversity since the focus was on method development.
greatly enhance the ease with which the mobility and retention of phosphite in plant tissues may be monitored.

REFERENCES

1. Loening, K. 1997. IUPAC consultant, Topterm, PO Box 21213, Columbus, Ohio, USA, Personal communication.


19. Capewell, S. Chemistry Department, Murdoch University, Murdoch 6150, Australia. Private communication. These results, along with related other systems, will be published elsewhere.

5.1.2 The problems with phosphite analysis

Doug Clarke and Jason Maroudas

Initially this analysis was performed on a resin based anion exchange column at a pH of 3.5. After about 40 samples had been analysed the column lost efficiency which resulted in loss of separation between the two peaks and it was found very difficult, if not impossible, to regenerate the column. It was then realised that the samples contained tannins (polyphenols) which have a similar structure to the styrene/divinyl based resin of the column. This means that it would be very difficult to remove these materials from the column. It was then decided to try a similar column but with a silica backbone which turned out to be quite successful. At this stage problems were encountered with another peak overlapping with the phosphate which made quantitative analysis very difficult. It was considered probable that this interfering peak was a low molecular weight organic acid (formic or acetic) and this could be eluted from the column very quickly by reducing the pH of the mobile phase (this has the effect of producing the molecular species of the acid rather than the ionic form).

Even with the silica-based column (Vydac 302IC4.6), it was found that the polyphenols present in the samples still created problems and in particular with those samples (root) which contained large amounts of tannins. However, by regenerating the columns with water, methanol, chloroform, methanol and water, the problem was initially solved. When this combination of solvents was still insufficient to clean the columns effectively it was found that using a high concentration of succinic acid buffer solution (200 mM) would often complete the regeneration procedure. A high concentration of buffer solution results in the rapid elution of material from the column and is a standard procedure. On one occasion a column could not be regenerated by any of the foregoing methods. It was then realised that the samples under test at this time were very high in phosphate content and because the buffer solution contained lithium hydroxide (used to adjust the pH of the solution) it was deduced that lithium phosphate had precipitated out which was causing a blockage in the column. In fact it was the high pressure generated by the system that had been the problem in this case. Since lithium phosphate is soluble in acetone this solvent was used in the regeneration procedure and found to be successful.

The problem of precipitated lithium phosphate was overcome by the use of sodium hydroxide rather than lithium hydroxide in the preparation of the succinic acid buffer and no further problems occurred because of precipitation.

It would also appear that the columns, when purchased, were not necessarily identical and some columns deteriorated more rapidly than others as well as appearing to have different characteristics. Eventually, there were four or five columns which could not be regenerated further by normal procedures and it was decided to remove the stationary stage from each column and combine all the material in an attempt to regenerate it using more stringent conditions. Concentrated nitric acid was used for this purpose and certainly removed large quantities of very dark brown material. The columns were then repacked at 4000 to 5000 psi and two columns of original specifications were prepared. It was considered
too dangerous to both equipment and personnel to use this method in the normal regeneration procedure. It is remarkable that the columns can withstand such harsh conditions as concentrated nitric acid and it is possible that this could not be repeated many times.

Without an effective procedure for removing tannins from the samples before analysis the problem of frequent regeneration of columns would always be present but although numerous methods have been attempted to this end, none have been successful. The principle reason for this is that any ‘clean up’ procedure always reduces the phosphite/phosphate concentration of the solutions which makes it impossible to perform a good quantitative analysis. However, this is a good analytical procedure provided there are sufficient funds to purchase three to four columns a year (approximately $500/column) and in any future costing of the project this should be taken into account. Added to that a number of pre-column purchases are also necessary.

If funds had been available the next step in this procedure would be to reduce practical detection limits from say 5 ppm to 0.5 ppm by means of suppressed ionisation techniques. These methods increase the sensitivity of detection by reducing the conductivity of the buffer solution before entering the detector which automatically allows for a more sensitive setting to be used on the conductivity meter.

Recommendations

- If HPIC is to be used to detect phosphite in plant tissue the HPIC unit, computer control and automatic injector need to be updated (approximate cost $40,000– $50,000). An alternative would be to manually inject each sample and this would reduce the cost to update to $10,000–15,000. However, a technician would be required to manually inject a sample every 50 minutes.
- There is a need to detect lower phosphite concentrations in plant tissue. This may be facilitated by the use of a suppressed ionisation column that may reduce the conductivity of the buffer solution, which would reduce baseline conductivity and allow the detector to be adjusted to a more sensitive setting.

Recommendations for future research

- Use of HPIC to detect phosphite in plant tissue needs to be refined to allow routine analysis of large numbers of plant samples.
5.2 Operational spray

Abstract

Use of firefighting equipment was a more rapid and practicable method than backpack spray units to spray small areas of jarrah forest with phosphite. In dense vegetation, two people sprayed $\frac{1}{10}$ of a hectare in 40 minutes and less dense vegetation was sprayed in 30 minutes. It is useful to include a dye in the spray solution to ensure that all of the vegetation is treated. The trees on the treated sites were injected with phosphite using a hydraulic sidewinder injector. One person was able to drill and inject 49-55 holes/hr, depending on the weather and the density of the understorey. To determine the time it would take to inject trees in an area the basal diameter of the trees needs to be estimated and divided by 20 cm (the distance between injection holes) and the number of injections can be determined.

Introduction

Application of phosphite using backpack spray units is slow and hard physical work, especially if the site to be treated is in dense vegetation. An alternative method using firefighting equipment was tested to determine whether it was effective for treating small areas under operational conditions. The overstorey component of the jarrah forest that could not be sprayed from the ground was trunk injected. Activities were timed to determine the approximate costs of treating small ($\frac{1}{10}$ of a hectare) areas of jarrah forest with phosphite.

Methods

Two areas in the jarrah forest (Worsley Alumina) which contained active dieback fronts and vehicle access were treated with 5 g phosphite/L. The vegetation of the first site was extremely thick; *Bossiaea aquifolium* dominated the understorey, the midstorey was *Banksia grandis* and the overstorey was a mixture of *Eucalyptus marginata* and *Corymbia calophylla*. The second site was more open; *Dryandra nivea*, *Xanthorrhoea* sp. and *Macrozamia riedlei* dominated the understorey, *B. grandis* dominated the midstorey and *E. marginata* and *C. calophylla* were the main species in the overstorey. The area of dieback at each site was mapped prior to treatment so that the areas could be revisited in the future to determine the long-term efficacy of phosphite.

The plants were sprayed to run-off in December 1998 (summer) with 5 g/L Foli-R-Fos 400 (and 0.25% phosphite) using a 30 metre hose (30 mm diameter) connected to a 500 L tank and pump (fire fighting pump), loaded on the back of a trailer. A nozzle approximately 5 cm in diameter with holes 0.25 mm in diameter (spaced 1 mm apart) was attached to the hose. In order to determine how effective the spray system was, a red dye (Redye, Crop Care Australasia Pty Ltd) was added to the phosphite solution to colour the vegetation that had been treated. The tank was filled with water, the phosphite and red dye
were added and the chemicals were thoroughly mixed by pumping the solution back into the tank for 10 minutes. The person who sprayed the plants walked at right angles to the road, into the bush spraying to one side and walked out on the same path spraying the other side. The spray was able to reach approximately 8 m from the nozzle therefore a line was made into the bush every 13 m to allow for overlap. The volume of phosphite used, area treated and time to treat this area was recorded.

Plants taller than 3 m were injected with phosphite (Foli-R-Fos) using a Sidewinder hydraulic tree injector (Sidewinder Injection Technology Pty Ltd, Queensland). Holes were drilled every 20 cm around the circumference of the tree at waist height (approx. 1 m from ground level). The holes were drilled to a depth of 2-3 cm using a 6 mm drill bit. Each hole was injected with 20 mL of 75 g phosphite/L. The number of holes drilled and injected and the time taken was recorded.

At the second site, 10 Banksia grandis and Trymalium ledifolium stems (30 cm long), were sampled randomly nine days after the plants were sprayed and analysed for phosphite using HPIC (Roos et al. 1999).

**Results**

It took two days to treat the first site. On the first day each person injected an average of 52 holes/hr. The second day was overcast and the injection rate at the first site was slower with each person treating an average of 49 trees/hr. The second site was also treated on the second (overcast) day. At the second site, which was more open than the first site, an average of 55 holes/hr were drilled and injected.

Two people were required to spray the vegetation; one person to hold the hose and another person to prevent the hose from getting tangled on the vegetation. At the first site it took 2 people 45 minutes to spray 500 L of 5 g phosphite/L and an area of 1 036 m² was treated. Thus, in dense understorey vegetation, two people would be able to spray approximately 1 000 m² of vegetation in 45 minutes and they would use nearly 500 L of phosphite solution. At the second site it took 2 people 30 minutes to spray 500 L of 5 g phosphite/L and an area of 1196 m² was treated. Thus, in more open vegetation two people would be able to treat 1 000 m² of vegetation in 25 minutes and they would use 418 L of phosphite solution. The red dye was helpful in determining which areas had been sprayed.

The average phosphite level in the Banksia grandis stems was 61 (SE±32) µg/g dry weight. Phosphite was not detected in two stems and levels ranged from 17-207 µg/g dry weight in the remaining B. grandis stems tested. The average phosphite level in Trymalium ledifolium stems was 110 (SE±34) µg/g dry weight. Phosphite was detected in all T. ledifolium stems and the range of phosphite levels was 11-370 µg/g dry weight.
Discussion

Use of fire fighting equipment was a relatively rapid method (compared with application using backpack units) for spraying areas that have access to roads or tracks. Using this equipment 1/10 of a hectare of dense vegetation was treated by two people in 40 minutes and more open vegetation was sprayed in 30 minutes. These times did not include the time taken to set up the equipment, drive to the site and mix the phosphite solution. It is also recommended that a dye be used in the spray solution to ensure that all vegetation is sprayed.

The average phosphite levels in the B. grandis stems were similar to the average levels found in B. grandis that had been sprayed using backpack spray units in autumn (mean=53, SE±17 µg phosphite g$^{-1}$ dry stem) or spring (mean=66, SE±20 µg phosphite g$^{-1}$ dry stem) (section x). This shows that the use of fire fighting equipment to spray plants is as effective in delivering the chemical to the plant as backpack spray units.

It is difficult to estimate the time needed to inject trees on a site as it depends not only on the number of trees present but also on the girth of the plants. If the approximate basal diameter of tree stems can be estimated this number can be divided by 20 cm (the distance between injection holes) and the number of injections can be determined. It is important to inject trees when the sun is shining, as the plants need to be actively transpiring to take-up the chemical (Ian Colquhoun, pers. comm.). It is also important not to spray plants when rain is expected within at least 7 hours as the chemical is washed from the leaves before it can enter the plant (Komorek et al. 1997).

Recommendations

- Use of fire fighting equipment is an effective method of spray application if road access is available.
- Red dye in the spray solution is an effective method to mark the areas that have been treated.
- These sites should be revisited to determine the efficacy of phosphite over time.
- Only small areas (< one hectare) can be treated using this method.

Recommendations for future research

- To determine the time between spraying plants and rainfall for a range of plant species to ensure that the optimum amount of phosphite is taken-up by the plants.
Chapter 6

Associated studies

A large project such as this one funded by the Australian Research Council and the Minerals and Energy Research Institute of Western Australia is able to support associated ‘satellite’ activities conducted by PhD students. Such studies are able to provide additional knowledge to a problem or question(s) that would otherwise not be conducted under the auspices of a large project with specific objectives in mind. In addition, it is unlikely that such PhD projects would be conducted in the absence of a large research project such as the present one. The benefits of such associations are that students get relevant industry related research training and experience, and in turn industry grants obtain additional benefits from more detailed studies that would otherwise not have been conducted.

The present project had 3 ‘satellite’ PhD projects associated with it. These examined the following:

1. The effect of phosphite on sexual reproduction on non-native and native plant species of the Eucalyptus marginata forest.
2. The effect of phosphite on ectomycorrhizal fungi.
3. The distribution of phosphite in Eucalyptus marginata after foliar phosphite treatment.
6.1 The effect of the fungicide phosphite on sexual reproduction of non-native and native plant species of the *Eucalyptus marginata* forest.

Meredith Fairbanks

A three-year study was conducted on Alcoa's Jarrahdale minesite on the effects of phosphite on reproduction of 3 perennial species that occur in the jarrah forest. Phosphite was applied at concentrations of 0, 2.5, 5 and 10 g/L in autumn, winter and spring of 1997 - 1999. Pollen fertility and seed germination was periodically tested for up to two years after phosphite application. From these studies, it was found that phosphite influences plant species differently, depending upon their life cycle and when they flower in relation to the season of spraying.

Phosphite at 2.5 g/L and above significantly reduced *Dryandra sessilis* pollen fertility when plants were sprayed in autumn and winter, with pollen germination being affected up to one year after spraying. Pollen fertility was not affected after the spring spray possibly due to the time duration between spraying and when the plant flowered. Seed germination was not affected.

The pollen fertility of *Trymalium ledifolium* was depressed by 2.5 g phosphite/L or greater when plants were sprayed in autumn and winter. When plants were sprayed in spring only 10 g phosphite/L depressed pollen fertility (Table 6.1). Seed germination was increased when they were collected 30 weeks after plants were sprayed with 2.5 g phosphite/L in winter and decreased when they were collected 35 weeks after the autumn spray (Table 6.1).

Table 6.1 Maximum duration in weeks of a significant effect (p<0.05) on pollen fertility or seed germination by sprays of (a) 2.5, (b) 5 or (c) 10 g phosphite/L.

<table>
<thead>
<tr>
<th>Year of phosphite application</th>
<th>Winter spray</th>
<th>Spring spray</th>
<th>Autumn spray</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pollen assessment</td>
<td>Seed assessment</td>
<td>Pollen assessment</td>
</tr>
<tr>
<td>1997</td>
<td>10 (a, b, c)</td>
<td>30 (a)</td>
<td>no effect</td>
</tr>
<tr>
<td>1998</td>
<td>no effect</td>
<td>no effect</td>
<td>38 (c)</td>
</tr>
<tr>
<td>1999</td>
<td>no effect</td>
<td>no effect</td>
<td>no effect</td>
</tr>
</tbody>
</table>

Phosphite at 2.5 g/L and above depressed pollen fertility of *Lasiopetalum floribundum* when sprayed in spring but not when it was sprayed in autumn or winter. As yet there are no results for seed germination for this species.

In another experiment, undertaken in a glasshouse at Murdoch University, on the annual *Pterocheata paniculata*, it was shown that phosphite had no effect on plants sprayed in the vegetative stage, but when sprayed at flower initiation there was a reduction in pollen germination at 2.5 g phosphite/L and above. Seed germination was reduced by 5 g phosphite/L and plants sprayed with 10 g phosphite/L died due to severe phytotoxicity.
Phosphite has been found to affect annual plants much more severely than the perennial species. In all species pollen fertility was affected by phosphite concentrations below that of the suggested operational rate (5 g phosphite/L). It appears that even though pollen fertility is affected this effect did not influence seed germination in the perennials. While in the self-pollinating annual species studied phosphite treatment also reduced seed growth.

A paper entitled ‘Comparisons of *Eucalyptus calophylla* tissue phosphite concentrations after spray, mist or soil drench applications with the fungicide phosphite’ has been accepted for publication by Australasian Plant Pathology (Appendix 1).

**Recommendations for future research**
- More studies are required on the effect of phosphite on plant reproduction.
6.2 The effect of phosphite on ectomycorrhiza

Kay Howard

Both ectomycorrhizal (ECM) and arbuscular mycorrhizal associations occur in many native plants. There have been reports of phosphite having detrimental effects on arbuscular mycorrhizae, while there are no reports in the literature of the effect on ECM. Therefore, it is important to determine if phosphite will have any effect on the formation and the persistence of ECM in native plant species. This was investigated by examining (i) the formation of the fungal mantle and Hartig net that surrounds the host root (ii) enzyme activity within ECM associations and in fungal tissue (iii) ability of spores to germinate and infect (iv) formation of ECM in soils naturally infested with ECM and the persistence of ECM when phosphite is applied.

In glasshouse trials there was no significant effect on ECM formation in *Eucalyptus marginata*, *Eucalyptus globulus* or *Agonis flexuosa* when phosphite was applied to the foliage at the recommended rate (5 g/L). Also, there was no significant effect on continued ECM colonisation by established *Pisolithus*, *Descolea* and *Scleroderma/E. globulus* mycorrhizae.

Enzymic studies on axenic cultures of *Pisolithus*, *Scleroderma* and *Phytophthora cinnamomi* showed that the enzyme activity in the hyphae of ectomycorrhizal fungi did not significantly change when *E. marginata* clones were treated with 3 g phosphite/L.

There was no significant effect of phosphite on the *in vitro* infection of axenic *E. marginata* by *Pisolithus* and *Scleroderma* spores when the plants were treated with 3 g phosphite/L.

It was found that the type of soil makes a significant difference to root and shoot phytotoxicity of *E. globulus* seedlings. When plants were grown in gravelly soil there was a significantly higher survival rate when the soil was drenched with 25 g phosphite/L (2.5 g phosphite/pot) compared with plants grown in sand.

While phosphite does not appear to have a great impact on ECM fungi there are many aspects not yet examined. For example, the effect of phosphite on the symbiosis in detail and over time is not known. The effect of phosphite on the strength of the association between the host and ECM fungi and host/mycorrhizal recognition factors needs to be ascertained. Furthermore, as phosphite can change root exudates and potentially change soil microbe populations it is important to determine if these changes influence ECM competition and succession.

**Recommendations for future research**

- Determine if there are long-term effects of phosphite on ECM/host associations.
6.3 Phosphite distribution in *E. marginata* after foliar treatment with phosphite

R. A. Pilbeam

**Methods** *E. marginata* seedlings in a rehabilitated bauxite mine pit were sprayed to run-off with 0, 5 or 10 g phosphite/L in early January. Plants were harvested one week later and separated into four tissue types: lower stem, lignotuber, tap root and lateral roots. Each sample was analysed for phosphite content using the HPIC method described by Roos et al. (1999). There were 6 replicate plants for each treatment.

**Results** No phosphite was detected in plants treated with zero phosphite.

**Table 6.2:** Phosphite concentration (µg/g dry wt) detected in various tissue types of *E. marginata* seedlings 7 days after foliar treatment with 5 or 10 g phosphite/L.

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>5 g phosphite/L</th>
<th>10 g phosphite/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower stem</td>
<td>209 ± 41</td>
<td>423 ± 48</td>
</tr>
<tr>
<td>Lignotuber</td>
<td>54 ± 13</td>
<td>110 ± 31</td>
</tr>
<tr>
<td>Tap root</td>
<td>28 ± 10</td>
<td>36 ± 10</td>
</tr>
<tr>
<td>Lateral roots</td>
<td>86 ± 19</td>
<td>68 ± 16</td>
</tr>
</tbody>
</table>

The lower stem was the only tissue type in which treatment with 10 g phosphite/L resulted in significantly (p=0.05) more phosphite than treatment with 5 g phosphite/L. Plants treated with 10 g phosphite/L had twice as much phosphite detected in the lower stem and lignotuber, yet the higher treatment resulted in slightly less phosphite in the roots overall (Table 6.2).

**Conclusion** There was no dosage effect on the concentration of phosphite detected in the roots of *E. marginata* 7 days after treatment with 5 or 10 g phosphite/L. A significant dosage effect was only apparent in the stems. Levels of phosphite in the stems were 2 and 4 times greater than in the roots of plants treated with 5 and 10 g phosphite/L, respectively.

Subsequent to this experiment, this student changed direction in her studies, so no further phosphite analysis was conducted for her.
List of recommendations

Chapter 2. Long-term effectiveness of phosphite to control Phytophthora cinnamomi

2.1 The long-term ability of phosphite to control Phytophthora cinnamomi in two native plant communities

Recommendations
- Vegetation may be treated with phosphite in spring or autumn.
- A single target species cannot be used to determine the appropriate time for reapplication of phosphite.
- To maintain protection of all plant species tested, phosphite needs to be reapplied every 6-12 months.
- Foliar application of 5 g phosphite/L is recommended to provide protection to plants and to minimise the effects of phytotoxicity.

Recommendations for future research
- To examine biochemical defense mechanisms in different hosts and the influence of in planta levels of phosphite.
- To investigate ways of increasing the uptake of phosphite.
- To develop more sensitive methods of phosphite detection in plant tissues.
- To determine how and where phosphite is moved and stored in the plant with seasonal changes.
- To develop methods that are rapid and cost effective which allow industry and land managers to determine when it is necessary to reapply phosphite to maintain its effectiveness.

2.2 The long-term ability of phosphite to control Phytophthora cinnamomi in five plant species native to Western Australia, in a glasshouse trial

Recommendations
- A single target species cannot be used to determine the time for the reapplication of phosphite.
- If underbark inoculation is to be used as a bioassay for the levels of induced resistance from phosphite application, plants must be inoculated at a time of year when they are susceptible to P. cinnamomi.

Recommendations for future research
- The mechanisms of phosphite uptake by different plant species need to be determined.
- The effects of temperature on the efficacy of phosphite both in vitro and in planta need to be determined.
2.3 The long-term prevention of death of jarrah forest plant species treated with phosphite and inoculated with *Phytophthora cinnamomi*

**Recommendations**
- Vegetation that is sensitive to phytotoxicity caused by phosphite should be sprayed earlier than November to avoid drought stress and allow time for them to recover before summer.

**Recommendations for future research**
- To determine the long-term efficacy of phosphite in preventing death of plants that are continually being challenged by *P. cinnamomi*, experimental plots should be set up in naturally infested areas.

**Chapter 3 Biology of phosphite-*Phytophthora cinnamomi* and host plant interactions**

3.2 Sensitivity of *Phytophthora cinnamomi* isolates to phosphite *in planta*

**Recommendations**
- Care should be taken when extrapolating results from *in vitro* to *in planta* and from the glasshouse to the field.
- Phosphite must be reapplied every 6 months to *E. marginata* seedlings growing in a rehabilitated minepit to maintain protection.

**Recommendations for future research**
- Investigate methods such as multiple applications of phosphite or different adjuvants to increase the uptake of phosphite by plants.
- Investigate the mechanisms of phosphite uptake in a range of plant species.

3.3 The effect of phosphite on *Phytophthora cinnamomi* zoospore production *in planta*

**Recommendations**
- Phosphite reduces but does not prevent the production of zoospores therefore, dieback hygiene measures must be maintained in areas that have been treated.

**Recommendations for future research**
- Investigate the ability of zoospores produced from phosphite treated plants to infect intact plants.
- Determine if zoospores can infect phosphite treated plants in the native vegetation.
The efficacy of phosphite in reducing the infection and colonisation of the roots of a range of Western Australian native plant species by *Phytophthora cinnamomi*

**Recommendations for future work**

- To determine the effect of temperature on the efficacy of phosphite in preventing growth of *P. cinnamomi*.
- To determine if phosphite is effective in reducing *P. cinnamomi* colonisation in *Xanthorrhoea preissii*.

Chapter 4 Possible deleterious effects of phosphite

**4.1 The effect of phosphite concentration on phytotoxicity in a range of plant species at Tiwest**

**Recommendations**

- Foliar application of 5 g phosphite/L should be used to minimise phytotoxicity symptoms.

**Recommendations for future research**

- More work needs to be conducted on the long-term effects of phosphite at different rates on the fitness of plants as affected by pests, disease and adverse environmental conditions such as drought.
- More work needs to be done on the effects of phosphite on flowering and pollen and seed viability.

**4.2 The effect of phosphite on seed production and seed viability**

**Recommendations for future research**

- Select key perennial and annual species and examine seed production and germination after phosphite treatment in plants treated with phosphite before, during or after anthesis.
- Develop assays other than the use of Tetrazollum to examine seed viability.

Chapter 5 Technical issues and operational spray

**5.1.2 The problems with phosphite analysis**

- If HPIC is to be used to detect phosphite in plant tissue the HPIC unit, computer control and automatic injector need to be updated (approximate cost $40 000– $50 000). An alternative would be to manually inject each sample and this would reduce the cost to update to $10 000- 15 000. However, a technician would be required to manually inject a sample every 50 minutes.
- There is a need to detect lower phosphite concentrations in plant tissue. This may be facilitated by the use of a suppressed ionisation column that may reduce the conductivity of the buffer solution, which would reduce baseline conductivity and allow the detector to be adjusted to a more sensitive setting.
Recommendations for future research

- Use of HPIC to detect phosphite in plant tissue needs to be refined to allow routine analysis of large numbers of plant samples.

5.2 Operational spray

Recommendations

- Use of fire fighting equipment is an effective method of spray application if road access is available.
- Red dye in the spray solution is an effective method to mark the areas that have been treated.
- These sites should be revisited to determine the efficacy of phosphite over time.
- Only small areas (< one hectare) can be treated using this method.

Recommendations for future research

- To determine the time between spraying plants and rainfall for a range of plant species to ensure that the optimum amount of phosphite is taken-up by the plants.

Chapter 6 Associated studies

6.1 The effect of the fungicide phosphite on sexual reproduction of non-native and native plant species of the Eucalyptus marginata forest

Recommendations for future research

- More studies are required on the effect of phosphite on plant reproduction.

6.2 The effect of phosphite on ectomycorrhiza

Recommendations for future research

Determine if there are long-term effects of phosphite on ECM/host associations.
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Appendix 1

Comparisons of phosphite concentrations in Eucalyptus calophylla tissues after spray, mist or soil drench applications with the fungicide phosphite.

M.M. Fairbanks, G.E.St.J. Hardy and J.A. McComb
(Accepted for publication by Australasian Plant Pathology)

Abstract
The fungicide phosphite was applied to four and eight month old Eucalyptus calophylla (marri) seedlings, by spraying to run-off with 0.25, 0.5 and 1% phosphite (2.5, 5 and 10 g/L ai, respectively), misting with 10, 20, and 40% phosphite (100, 200 and 400 g/L ai, respectively) or applying a 1% phosphite (10 g/L ai) soil drench. The phosphite concentrations in plant tissues were determined by High Performance Ion Chromatography analysis, 7 days after treatment. Phosphite concentrations found in the plant tissues were higher than previous published results. Phosphite concentrations were generally higher in the root tips than in mature roots, and in shoot tips compared to stems and leaves. Highest concentrations were recorded in root tips of soil drenched plants. When phosphite concentrations in shoot apices were compared, spray to run-off at 0.5% gave a comparable concentration to a 10% mist treatment and the soil drench, while a 1% spray was comparable to the 20% and 40% mist treatment. When phosphite concentrations in root apices were compared, spray to run-off at 0.5% and 1% gave comparable concentrations to a 10 or 20% mist treatment. All treatments except 0.25%, 0.5% spray and soil drench caused some phytotoxicity on the foliage.

Introduction
In the south-west of Western Australia, the soil-borne plant pathogen Phytophthora cinnamomi Rands is pathogenic to some 2000 of the 9000 native plant species (Wills 1993). The pathogen kills its host by destroying the roots and girdling the base of the stem depriving the plant of nutrients and water (Shearer et al. 1991; Shearer 1994). To date, control measures have included quarantine to reduce the spread of the disease, selection and micropropagation of resistant individuals, and the establishment of seed banks of rare and endangered plants susceptible to the disease. Recently however, the fungicide phosphite (also called phosphonate) has been shown to effectively contain the pathogen and in the case of some Banksia species, prevent plant deaths for up to 5 years when applied as a trunk injection (Shearer 1994).

Phosphite, the anionic form of phosphonic acid ([HPO₃]⁻²) provides a cheap and effective means of controlling P. cinnamomi in horticulture and native plant communities (Coffey and Bower 1984; Wicks and Hall 1988; Ouimette and Coffey 1989; Guest and Grant 1991; Shearer 1994). It is a systemic fungicide which is rapidly absorbed and translocated initially in the xylem and then in the phloem (Guest and Grant 1991).
Phosphite can be applied as a soil drench, by trunk injection, ground level or aerial foliar sprays (deBoer and Greenhalgh 1990; Holderness 1992). In the south-west of Western Australia, phosphite at a concentration of 0.5% is being used for spraying (to run-off) small areas of native vegetation threatened by *P. cinnamomi* on mine rehabilitation sites (Hardy pers. comm.). In addition, aerial application by misting of 40% phosphite is being evaluated by the Department of Conservation and Land Management (CALM) for control of *P. cinnamomi* in larger areas of the south coast and Northern Sandplains of Western Australia (Gillen and Grant 1997; Komorek and Shearer 1997; Barrett pers. comm.). An advantage of aerial misting is that large or inaccessible areas can be sprayed economically. This is particularly important for the protection of rare and endangered plant species.

### Table 1 Published data on the use of phosphite on plants and their subsequent concentration of phosphite in plant tissue

<table>
<thead>
<tr>
<th>Species</th>
<th>Application method</th>
<th>Phosphite (%)</th>
<th>Time of analysis (weeks)</th>
<th>Phosphite content (µg/g)</th>
<th><em>Ref.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Persea americana</em> (avocado)</td>
<td>Soil drench</td>
<td>0.21</td>
<td>8</td>
<td>213</td>
<td>382</td>
</tr>
<tr>
<td><em>Zea mays</em> (maize)</td>
<td>Soil drench</td>
<td>0.25</td>
<td>7</td>
<td>3070</td>
<td>5544</td>
</tr>
<tr>
<td><em>Banksia telmetia</em></td>
<td>Mist</td>
<td>10</td>
<td>1</td>
<td>5.2</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td>19.6</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td></td>
<td>91.3</td>
<td>115.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>26</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td></td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>52</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td>3</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td></td>
<td>6.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>


High Performance Ion Chromatography (HPIC) and gas chromatography have been used to measure the phosphite concentrations of plant tissue. Ouimette and Coffey (1989) used HPIC to analyse tissues of field-grown avocado after foliar and soil treatment with potassium phosphite. They found that 8 weeks after drenching there were high concentrations of phosphite in the root and stem tissue (Table 1). Similar results for maize in a pot trial, were obtained using gas chromatography, after soil drenching,
although after 7 weeks the tissue concentrations were 10 times greater than those reported in avocado (Seymour et al. 1994).

In a 2 year field study of phosphite misted Banksia telmetia A.S. George, Komorek and Shearer (1997) showed that after 1 week, phosphite concentrations were similar in roots and leaves, while at 6 months and especially after 1 year, phosphite was more concentrated in the roots. By two years, phosphite was not detectable in any tissue. Their study analysed phosphite with gas chromatography using a P-sensitive column and a flame photometric detector. The findings suggest that either maize accumulates higher levels of phosphite than Banksia or avocado, or that glasshouse conditions enable greater uptake (Table 1).

Given the different modes used to apply phosphite to plants and the subsequent wide range of tissue concentrations reported, it is important for comparative purposes to determine how the different application methods affect the tissue uptake within a species. In this paper we compare the phosphite concentrations in Eucalyptus calophylla (R.Br. ex Lindley) Hill and Johnson (marri) roots, shoots, leaves and stems after applying phosphite, with a low volume mist spray, spraying to run-off and by soil drench.

Methods

**Plant material**  Eucalyptus calophylla is a native broad-leaved co-dominant tree of the Eucalyptus marginata (jarrah) forest. Four-month old seedlings from a common seed-lot, were obtained from the Marrinup Nursery (Alcoa World Alumina - Australia Limited, Dwellingup, Western Australia). The experiments were conducted in an air-cooled glasshouse at Murdoch University. In the first experiment, 28 plants, with a mean height of 45cm, were potted into 7 L pots using Yates Macro blend potting mix (Arthur Yates and Co. Limited, NSW, Australia) and fertilised 6 weeks before phosphite applications with 15 g/pot of low phosphorous osmocote (Scotts Australia Pty Ltd). In the second experiment, 71 plants were grown in the same way before use. Plants were 8 months old at time of treatment, and had a mean height of 85cm.

**Phosphite Application**  In the first experiment, plants were either sprayed to run-off with 0, 0.25, 0.5 or 1% phosphite or misted for 4 seconds with 10, 20 or 40% phosphite (Foli-R-Fos 400 fungicide U.I.M. Agrochemicals (Aust) Pty Ltd. Qld, Australia, active ingredient 400 g/L phosphorus (phosphonic) acid present as the mono-di potassium phosphite), all treatments included 0.25% Synertrol oil (Organic Crop Protectants Pty Ltd. NSW, Australia) as a sticking agent. A 15 L capacity backpack sprayer and an Ulva Micron 2 L capacity mister (Micron Sprayers Limited, U.K. with a mist rate of 28 ml/min) were used for the spray to run-off and mist treatments, respectively. There were four plants/treatment.

In the second experiment, plants of 85 cm average height were sprayed to run-off or misted as above. There were seven plants/treatment. A further seven plants were soil drenched with each 7 L pot receiving 800 ml (field capacity of the pot) of 1% phosphite, care was taken not to wet the foliage. Soil was not protected during spraying. The soil-drenched plants were not watered until 24 h after treatment and, together with the foliar treated plants were hand watered to keep the leaves dry.
Phytotoxicity Rating Plants were rated for foliar phytotoxicity symptoms at the time of harvest, seven days after phosphite treatments. A rating system was devised that assessed the proportion of the leaf area affected (burnt) and how much of the plant was affected. For example, from Table 2, if the average area of each leaf burned was 15%, and this occurred over 25% of the plant, the phytotoxicity rating was calculated as \((0.15 \times 0.125)/100 = 1.875\%)\). Phytotoxicity ratings are presented only for the second experiment.

Table 2 Rating system for foliar phytotoxicity symptoms of phosphite in *Eucalyptus calophylla* plants treated in Experiment 2

<table>
<thead>
<tr>
<th>Proportion of leaf area damaged</th>
<th>Value used in calculation</th>
<th>Proportion of plant affected</th>
<th>Value used in calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10%</td>
<td>0.05</td>
<td>0- 25%</td>
<td>0.125</td>
</tr>
<tr>
<td>11- 20%</td>
<td>0.15</td>
<td>26 – 50%</td>
<td>0.375</td>
</tr>
<tr>
<td>21 – 50%</td>
<td>0.35</td>
<td>51 – 75%</td>
<td>0.625</td>
</tr>
<tr>
<td>51 – 75%</td>
<td>0.625</td>
<td>76 – 99%</td>
<td>0.875</td>
</tr>
<tr>
<td>76 – 100%</td>
<td>0.875</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>100% leaf drop</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Harvest Plants were harvested seven days after treatment. In order to remove surface deposits of phosphite from foliage and stems, all plant tissue was washed in phosphate free detergent (Palmolive, Colgate-Palmolive Pty. Ltd, Sydney) (2.5 ml detergent per 1 L of tap water) then rinsed twice for 20 sec in tap water, and once in de-ionised water. The plants were tipped out of the pots and root tips detached and washed in de-ionised water. The apical 3 cm of roots were removed for separate analysis. Plant parts were dried at 37°C for 6-12 days and then ground with a grinder (Brookedes Pty. Ltd. Morley, Western Australia) to 0.5 mm in preparation for phosphite analysis.

Phosphite Analysis In Experiment 1, root and shoot tips, mature roots, young fully expanded leaves and mature leaves were analysed for phosphite concentration using HPIC (Roos *et al.* 1999). In the second experiment, only the root and shoot tips were assessed, as these were shown from experiment 1, to have the highest concentrations of phosphite.

All four plants/treatment from Experiment 1 were analysed by HPIC for phosphite analysis, but for Experiment 2, four randomly selected plants from the seven replicates in each treatment were analysed. The phosphite levels in the root and shoot tips between the two experiments were not significantly different from each other. Therefore, the root and shoot tip results from each experiment were combined to give eight replicates of root and shoot tips per treatment.
**Statistical Analysis**  
Results are expressed as the mean and standard error of the mean for all variables studied. Means were compared by one-way analysis of variance. Where differences were obtained due to experimental treatments, Dunnett's test (Dunnett 1955) was applied with a significance level of 95%.

**Results**  
Due to leaf damage that occurred prior to the start of the experiment, control plants exhibited some leaf symptoms that were scored with the phytotoxicity rating system. Compared to the control plants, the phytotoxicity rating was not significantly ($P > 0.05$) affected by the 0.25% and 0.5% spray or the 1% soil drench. In contrast, the phytotoxicity ratings for the 1% spray and all misted plants were significantly ($P = 0.04$) higher than the control (Figure 1).

![Figure 1](image)

Figure 1 Mean phytotoxicity rating and standard errors of *Eucalyptus calophylla* 7 days after being treated with phosphite by spraying (0, 2.5, 5, 10 g L$^{-1}$ open bar), misting (100, 200, 400 g L$^{-1}$, spotted bar) or soil drenching (10 g L$^{-1}$, solid bar) (Experiment 2 results).

The highest phosphite concentration within the plant was generally found in either the root tips or shoot tips. This is statistically significant except for the 0.5% spray to run-off treatment. In general, as the phosphite concentration applied increased so did the tissue concentrations of phosphite (Table 3). This is also true for the phosphite concentrations in the shoot tips of the misted plants and the shoot and root tips of the sprayed plants in Experiment 2 (Figure 2).
Figure 2 Mean phosphite content and standard errors of *Eucalyptus calophylla* shoot tips and root tips 7 days after being sprayed (0, 2.5, 5, 10 gL$^{-1}$), misted (100, 200, 400 gL$^{-1}$) or soil drenched (10 gL$^{-1}$) with phosphite. Data for spraying and misting are from Experiments 1 and 2 combined, data for soil drenching are from Experiment 2. Note the root tip concentration after soil drenching is off scale.

The root tips of the sprayed plants appeared to have a higher phosphite concentration than in the shoot tips but the difference was not statistically significant. In the misted plants, the phosphite concentration in root tips was more similar to that in shoot tips.

The root tips of plants that were soil drenched had the highest phosphite concentration 41 095 µg/g of all the treatments. In contrast, the phosphite concentration of the shoot tips was 1611 µg/g, similar to that of plants which received the 0.5% spray or the 10% mist (Figure 2).
Table 3 Mean phosphite concentrations in mature roots, stems, mature leaves and young fully expanded leaves in *Eucalyptus calophylla* 7 days after being sprayed (0, 0.25, 0.5, 1%) or misted (10, 20, 40%) with phosphite (Experiment 1 results only).

* Mean shoot and root tip data from Experiment 1 and 2 combined

<table>
<thead>
<tr>
<th>Phosphite concentration applied to plant (%)</th>
<th>Mean phosphite content of tissue (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root Tips*</td>
</tr>
<tr>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>0.25% spray</td>
<td>1813</td>
</tr>
<tr>
<td>0.5% spray</td>
<td>2205</td>
</tr>
<tr>
<td>1% spray</td>
<td>3561</td>
</tr>
<tr>
<td>10% mist</td>
<td>789</td>
</tr>
<tr>
<td>20% mist</td>
<td>2622</td>
</tr>
<tr>
<td>40% mist</td>
<td>1355</td>
</tr>
</tbody>
</table>

Discussion

Concentrations of phosphite within marri were in general, higher in the growing shoot tips or root tips, than in the more mature parts of the plant. Phosphite was at the highest concentration in the root tips of marri, 7 days after being soil drenched with 1% phosphite. Our results suggest an accumulation of phosphite in root tips after spraying, but not misting and the difference between roots and shoot tips are not statistically significant. Komorek and Shearer (1997) who analysed whole roots of *Banksia* showed a strong phosphite accumulation in roots 26 weeks after treatment of plants.

The concentration of phosphite found in marri roots and shoots (Table 3, Figure 2) were considerably higher than those reported previously (Table 1). This may be because of species, leaf structure, size of the plants, time or method of analysis. In addition, the root/shoot ratio of plants grown in pots may differ significantly from those grown in the field and the high levels in the roots in the experiment using pot grown maize and marri may be a reflection of the smaller root mass of pot grown plants.

All misting levels tested (10 to 40%) and spraying to run-off with 1% phosphite caused similar levels of phytotoxicity, but in marri the damage was not sufficient to kill the plant. The soil drench resulted in a very high tissue concentration of phosphite without tissue damage.

The operational concentration of phosphite application on Western Australian native vegetation is 0.5% for spraying to run-off and 40% for misting. These concentrations are chosen as higher levels result in severe phytotoxicity, and some plant death after treatment (Hardy unpublished results, Shearer pers. comm.). We have shown that the phosphite concentration in marri shoot tips sprayed to run-off with the operational rate of 0.5% was equivalent to the 10% mist, while the 1% spray to run-off resulted in a
phosphate concentration equivalent to the 20 and 40% mist. When the phosphate concentrations in marri root tips were compared, 0.5% spray to run-off was comparable to the 20% mist.

**Acknowledgments**

We thank Alcoa World Alumina – Australia Limited for supplying the plants, Jason Maroudas for conducting the phosphate analysis, Janet Holmes and Karen Brown of Murdoch University for technical assistance.

**References**


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'Operational' spray of phosphite in jarrah forest at Worsley Alumina (see Chapter 5.2)

Back-pack foliar application of phosphite at Iluka Resources, Eneabba (see Chapter 2.1)

Phytotoxicity symptoms after foliar application of 20 g phosphite/L (2%) at Tiwest, Cataby (see Chapter 4.1).
Phytophthora cinnamomi lesion development in Banksia grandis treated with (a) 5, (b) 0 and (c) 10 g phosphite/L. The arrows mark the top and bottom of the lesion (see Chapter 2.3).

Field trial examining the effect of foliar application of phosphite on zoospore production from infected Eucalyptus marginata seedlings. Buckets were used to simulate ponding around the base of the plants. Alcoa World Alumina, Jarrahdale (see Chapter 3.3).

Aeroponics system showing root development of Pattersonia occidentalis prior to inoculation with Phytophthora cinnamomi zoospores (see Chapter 3.4).