

PHOSPHITE AND ITS POTENTIAL TO CONTROL *P. CINNAMOMI* IN NATURAL PLANT COMMUNITIES AND ADJACENT REHABILITATED MINESITES IN WESTERN AUSTRALIA

G E. St. J. Hardy

School of Biology and Biotechnology, Division of Science and Engineering, Murdoch University, Murdoch 6150, Western Australia

SUMMARY

To date the majority of work on the systemic fungicide phosphite (phosphonate) to control *Phytophthora* diseases has been conducted on horticultural crops. There is a paucity of work on the control of *Phytophthora* root and collar rots in natural plant communities. This paper gives an overview of studies conducted in Western Australia

which examine the potential of using phosphite in plant communities rich in diversity and heavily impacted by *Phytophthora cinnamomi*. Details are given on possible beneficial and detrimental effects of using phosphite in natural plant communities to control *P. cinnamomi*.

INTRODUCTION

Phytophthora cinnamomi is a widespread and devastating plant pathogen in the south-west of Western Australia. It affects horticulture, mining, forestry and natural plant communities (Colquhoun, 2000; Hardy, 2000; Tommerup et al. 2000). Spot infections (< 1ha) in the *Eucalyptus marginata* (jarrah) forest or banksia woodlands and heathlands to be mined (alumina or mineral sand mines) cause operational problems for mining companies. In addition, new spot infections in the forest or heathlands caused by mining and other users are a threat to adjacent uninfested forest and heathlands. These 'spots' may be as small as one or two individual plants and do offer managers the opportunity to use systemic fungicides to minimise the risk of spreading the fungus, and to conserve trees and understory plants in the infested areas. The development of a method to contain or eradicate *P. cinnamomi* in such sites will help mining companies and managers of natural plant communities (National Parks, State Forests or Reserve Lands) meet their objectives of minimising the spread of the pathogen and to help financially reduce costs associated with hygiene.

The phosphite fungicides control many plant diseases caused by *Phytophthora*, even at concentrations *in planta* that only partially inhibit pathogen growth *in vitro* (Guest and Bompeix, 1984; Guest and Grant, 1991.). They are unique among fungicides in that they are translocated in both the xylem and the phloem (Ouimette and Coffey, 1989). In the phloem, phosphite is trapped and therefore translocated through the plant in association with photoassimilates in a source-sink relationship (Saindrenan et al, 1988, Ouimette and Coffey, 1990; Guest and Grant, 1991). Photoassimilates and therefore phosphite concentrations are thought to be higher in regions of the plant undergoing rapid growth, such as the roots and shoots (Whiley et al. 1995). The phosphite concentration in plant tissues is directly related to its application rate (Smillie et al. 1989). Phosphite treatment induces a strong and rapid defense response in the challenged plant (Guest and Bompeix, 1990). These defense responses stop pathogen spread in a large number of hosts. Phosphite exhibits a complex mode of action, acting directly on the pathogen and indirectly in stimulating host defence responses to ultimately inhibit pathogen growth (Guest and Grant, 1991). Phosphite has also been shown to inhibit sporulation of *Phytophthora* spp. at low concentrations (Farh, et al, 1981). Elicitors and chemicals such as phosphite are known to activate the phenolpropanoid pathway, although phosphite only stimulated host defences, including the phenylpropanoid pathway, after pathogen challenge (Saindrenan et al. 1988; Nemesothy and Guest, 1990). If resistance of plants to *Phytophthora* spp. is increased and the pathogen in the phosphite treated tissue cannot reproduce, then potentially the pathogen could be eradicated from the treated areas.

Research activities in Western Australia

In Western Australia, early work conducted by the Department of Conservation and Land Management showed that phosphite could control *Phytophthora* spp. in *Banksia grandis*, *E. marginata* and rare and endangered *Banksia* spp. (Komorek and Shearer 1997; Shearer and Fairman, 1997). They have applied the fungicide as trunk injection, foliar spray to run-off, and from aircraft as ultra-low-volume or mist sprays. Although these studies looked promising, they had only been conducted on a limited number of native plant species mainly from the Proteaceae and on *E. marginata*. Therefore, we initiated a number of studies to examine the potential of phosphite to control *P. cinnamomi* in native plant communities in and adjacent to mining. These studies aimed to determine:

- rates of phosphite that could be safely used in native plant communities
- if phosphite could prevent multiple deaths in a range of native plant species,
- could minimise the spread of *P. cinnamomi* from infested into uninfested areas,
- the persistence of phosphite in plant tissues and its long-term ability to control *P. cinnamomi*,
- if phosphite could prevent the sporulation and release of zoospores of *P. cinnamomi* from contained lesions,
- if phosphite adversely influences the reproductive fitness of annual and perennial plant species,
- if phosphite is detrimental to beneficial mycorrhizal associations, and
- differences between foliar application to run-off and foliar application as a mist (low volume application) in the uptake of phosphite in plant tissues.

If phosphite is found to be effective and safe to use the outcomes of these studies will provide us with a set of practicable and economic procedures for the application of phosphite in natural plant communities and rehabilitated minesites. Such information would include details of application rates, frequency of application and season of application.

Rates of application

Our research shows that phosphite as a foliar spray to run-off can safely be applied to natural plant communities at 5 g L⁻¹ phosphite with 2.5 g L⁻¹ synetrol oil (Organic Crop Protectants Pty. Ltd. NSW, Australia) as a sticking agent and that this rate controls *P. cinnamomi* in plant tissues for at least 6 months. Higher rates of phosphite (10 g and 20 g L⁻¹) result in phytotoxicity symptoms in a range of plant species, causing defoliation and death of some plant species. In contrast, as a mist application, phosphite is routinely applied at 24 kg ha⁻¹ and controls *P. cinnamomi* for at least 2 years (Komorek et al. 1997). Since, the two methods of application (spray to run-off and mist or low-volume spray from aircraft) are being used, it was

decided to make a comparison of phosphite uptake between the two types of application. Eight month old *Eucalyptus calophylla* (marri) grown in an evaporatively cooled glasshouse were sprayed (i) to run-off with 2.5, 5 and 10 g L⁻¹ phosphite, (ii) misted with 100, 200 and 400 g L⁻¹ phosphite or (iii) as a 10 g L⁻¹ phosphite soil drench. The phosphite concentrations in plant tissues (root tips, shoot tips, mature roots, fully expanded leaves and mature leaves) were determined by High Performance Ion Chromatography (HPIC)(Roos et al. 1999), 7 days after the spray treatments. Phosphite concentrations were higher in the root or shoot apices than in other more mature parts of the plant. The highest concentrations were recorded in root tips of the soil-drenched plants. When the different foliar treatments were compared in the shoot apices, spray to run-off at 5 g L⁻¹ gave a comparable concentration to the 100 g L⁻¹ mist treatment, whilst a 10 g L⁻¹ phosphite spray to run-off was comparable to 200 or 400 g L⁻¹ phosphite mist treatment. A comparison of root apices revealed that spray to run-off at 5 and 10 g L⁻¹ gave comparable concentrations to a 100 or 200 g L⁻¹ mist treatment. All treatments except the 2.5 g L⁻¹, 5 g L⁻¹ and soil drench (10 g L⁻¹ phosphite) caused some phytotoxicity to the foliage.

The uptake and subsequent tissue (lower stem, lignotuber, tap root and lateral roots) distribution of phosphite in *E. marginata* growing in a rehabilitated minesite 7 days after being treated with 0, 5 or 10 g phosphite L⁻¹ to run-off showed that significantly more phosphite was taken up by plants treated with 10 than 5 g L⁻¹ phosphite to run-off (Pilbeam, unpublished). At 5 and 10 g L⁻¹ the lower stems contained more phosphite (209 and 423 µg g⁻¹ dry wt, respectively) than the other tissues. The lower stems contained more phosphite than the other plant tissues.

There are large differences in the uptake of phosphite and phytotoxicity in plants grown in the glasshouse and those growing naturally in natural plant communities. For example, after spray application of phosphite the concentrations of phosphite in glasshouse grown plants are much higher than those recorded in plants growing in the wild. Wilkinson et al (2000c) found that jarrah grown in the glasshouse and treated with phosphite contained 8 times more phosphite than jarrah in a rehabilitated minesite. The higher rates could be due to the higher relative humidity in the glasshouse compared to the rehabilitated mine sites. 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. (Fairbanks et al. (2000) found the levels of phosphite in glasshouse grown *Eucalyptus calophylla* 7 days after a 10g L⁻¹ phosphite treatment to run-off to reach 3561 and 2550 µg g⁻¹ in root tips and shoot tips, respectively. Also in the glasshouse, plants appear not to be so sensitive to the phytotoxic effects of phosphite as they are in the field.

Control of *Phytophthora cinnamomi*

Our research has shown that phosphite can contain the spread of *P. cinnamomi* in many native plant species from a range of susceptible families (Pilbeam et al, 2000a, b; Wilkinson et al, 1999; Barrett, unpublished). The pathogen disappears from the "walled off" lesions over time, but the rate at which it disappears depends on the isolate and the host. There appears to be a large variation in the survival and aggressiveness between isolates (Hardy, unpublished). However, when plants are inoculated 6-to-18 months after phosphite treatment, the ability of the fungicide to contain the pathogen is reduced. And in some plant species, 6 months after phosphite treatment, lesion development is slow but not halted in the stems of inoculated plants (Pilbeam et al, 2000b; Wilkinson et al, unpublished). This indicates that the pathogen is not being contained but rather slowed down in its ability to colonise host tissue, as a consequence it is likely that phosphite will need to be sprayed every 1-to-2 years.

In glasshouse studies (Wilkinson et al, 1997) showed that phosphite, when applied as a foliar spray, did not prevent *P. cinnamomi* sporulating from diseased tissue. Zoospores from this tissue were able to infect *Pimelea ferruginea* cotyledons, indicating that zoospores are capable of causing disease, this was despite the

pathogen being effectively contained within the plant. This study was repeated in a rehabilitated minesite on 1-2 year old jarrah (Wilkinson et al, 1999). The stems of the jarrah were underbark inoculated and lesions were allowed to develop for approximately 7 days. The plants were then treated with phosphite as a foliar application, buckets were then attached to the stems below the lesions and the inoculated area of the stem was flooded. *Pimelea ferruginea* cotyledons were placed in the water and plated regularly onto a *Phytophthora* selective medium. A fine mesh was placed around the stems to ensure the leaves did not touch the stems and become infected through mycelial contact. In addition, aliquots of water were collected regularly and plated onto the *Phytophthora* selective medium. Once again, infected and phosphite treated plants were able to produce zoospores. Therefore, the treatment of infested sites may prevent death of plants but not necessarily prevent the spread of inoculum into non-infested areas. This observation also raises questions about the use of phosphite in container nurseries, where the pathogen may be controlled but not killed, and once plants are planted out the pathogen can be disseminated.

Effects on ectomycorrhizal fungi

Preliminary work on ectomycorrhizal and endomycorrhizal fungi indicates that when phosphite is applied at recommended rates (5 g L⁻¹ foliar application to run-off) it has no detrimental effects *in vitro* or *in planta* on these symbiotic associations (Howard et al, 1999). In these studies a range of *Pisolithus tintorius*, *Scleroderma* spp., *Descolea* sp. and *Laccaria laccata* isolates were screened *in vitro* and *in planta* for sensitivity to phosphite. *In vitro*, growth of the isolates was stimulated by concentrations of phosphite that inhibits isolates of *P. cinnamomi*. *In planta*, phosphite application had no effect on ectomycorrhizal formation but did stimulate a four-fold increase in arbuscular mycorrhiza (AM) colonisation. However, at 10 g L⁻¹ phosphite did significantly decrease infection by *Descolea* (Howard et al. 2000). This is the first study to examine the effect of phosphite on ectomycorrhizal fungi. However, there are conflicting results on the effects of phosphite on AM in annual species. For example, Jabahi-Hare and Kendrick (1987) observed an increase in AM in leek treated with phosphite, whereas Seymour et al. (1994) and Sukarno et al. (1996) found phosphite to decrease AM in maize and onion, respectively. In addition, spore germination and root infection were also not adversely affected by phosphite (Howard, unpublished data). Therefore, although only a few isolates from a small number of species were tested, it appears that phosphite used at recommended rates will not be detrimental to ectomycorrhizal fungi in natural plant communities.

Phytotoxicity symptoms and plant uptake of phosphite

Phytotoxicity is a major problem associated with the application of phosphite. There is a fine balance between rates of phosphite applied, phytotoxicity symptoms and the control of *P. cinnamomi*. In general, as the rates of phosphite applied increase so do the phosphite concentrations in plant tissues. However, above 5 g L⁻¹ (spray to run-off) or 36 kg ha⁻¹ (mist or low volume application) phosphite phytotoxicity symptoms increase substantially in a large range of species from different genera and families. These high rates can result in plant deaths, reduced growth, growth abnormalities and reduced reproductive capacity. There is also a large variation in uptake of phosphite and phytotoxicity symptoms between plant species and within individuals of a plant species. For example, Pilbeam et al. (2000) found that after the foliar application of phosphite to run-off, the foliage of naturally growing *Adenanthos barbiger* and *Daviesia decurrens* had mean phosphite concentrations of 80 and 871 µg g⁻¹ dry weight, respectively. In another study, also in a natural plant community, five weeks after phosphite application between 36 kg ha⁻¹ to 144 kg ha⁻¹, foliar phosphite concentrations varied from 1400-4500 ppm, 73-185 ppm, 124-402 ppm, 481-1055 ppm and 672-590 ppm for *Jacksonia spinosa*, *Adenanthos cuneatus*, *Melaleuca thymoides*, *Lysinema ciliatum* and *Banksia coccinea*, respectively (Barrett, in preparation). All of these species were

closely associated with each other and indicate the large differences between plant species in their uptake of phosphite under a given set of conditions. These species also varied in their sensitivity to phosphite as indicated by phytotoxicity symptoms. *J. spinosa* and *L. ciliatus* expressed the highest phytotoxicity ratings, whilst *B. coccinea* and *A. cuneatus* expressed the lowest phytotoxicity ratings. Overall, phytotoxicity increased with increasing levels of phosphite measured in the tissues. This again suggests that there are differences in the uptake of phosphite in these species. For example, *J. spinosa* is characterised by having stems that are in the form of green photosynthetic cladodes which may take up and retain higher phosphite concentrations than woodier stem material (Barrett, *pers. com.*). In addition, they do not shed leaves as a response to phytotoxicity, unlike many of the other species tested, which might also account for the high levels of phosphite measured *in planta*. Eleven macro- and micro-morphological leaf characteristics were assessed to determine which affected the phytotoxicity ratings. Growth form, leaf size, leaf orientation, position of veins relative to the leaf surface and mode of regeneration post fire (re-seeders or re-sprouters) did not influence phytotoxicity ratings (Barrett, in preparation). Whilst, plant height, leaf shape, leaf hairs, the distribution and position of stomata relative to leaf surface and the presence of oil glands significantly influenced phytotoxicity ratings. Phytotoxicity ratings were significantly higher in species with oil glands present and significantly lower in species which had stomata restricted to the lower surface and species with leaf hairs (Barrett, in preparation). In addition, the taller the plant species the greater the likelihood that phytotoxicity ratings would be high. The influence of stomatal characteristics on phytotoxicity ratings suggests that they may be important in phosphite uptake. The presence of leaf hairs also suggests that phosphite uptake is reduced by preventing effective spray contact, and droplet spread. Features such as cuticle thickness and epidermal cell size still need to be assessed for their effects on phosphite uptake and phytotoxicity (Barrett, *pers. com.*). There are large micro- and macromorphological differences of leaf characteristics between species within a genus that would account for the variation in phosphite uptake and effects of phytotoxicity. Growth abnormalities can also occur in a range of species from different families after phosphite treatment, these include 'little leaf' (Barrett, unpublished) and fasciation (Hardy, unpublished). Finally, in some species where phytotoxicity symptoms have been observed the incidence of aerial cankers can increase (Hardy, unpublished; Barrett, unpublished).

Effects on plant reproductive process

Pollen tube development and seed viability are definitely affected in the short-term by treatment with phosphite at recommended rates as foliar applications to run-off or as ultra-low volume foliar application (Fairbanks et al, 1998; Fairbanks et al, 1999). Phosphite influences plant species differently, depending upon their life cycle and when they flower in relation to the season of spraying. For example, phosphite at 2.5, 5 and 10 g L⁻¹ (recommended rate is 5 g L⁻¹) and above significantly reduced *Dryandra sessilis* (perennial species) pollen fertility when the plants were sprayed in autumn and winter, with pollen germination being influenced up to one year after spraying. Pollen fertility was not affected after a spring spray possibly due to the time duration between spraying and when the plant flowered. Seed germination was not effected. Similar observations were observed for other perennial species (Fairbanks, unpublished). In an annual species, *Pterochaeta 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 L⁻¹ and above. Seed germination was reduced by 5 g L⁻¹. Overall, phosphite has been found to affect annuals much more than perennials. In all species, pollen fertility was affected by phosphite concentrations lower than those recommended. It appears that in perennials despite pollen germination being affected seed germination was not. In contrast, in the annual species studied, seed germination is detrimentally

influenced. This affect is enhanced in self-fertilising species (Fairbanks, unpublished).

In another study, also conducted in natural plant communities, phosphite influenced flower production and fruit production when applied before flowering and during flowering. Fruit production was significantly inhibited in a number of plant species after low volume phosphite applications at 36, 72 and 144 kg ha⁻¹. At 144 kg ha⁻¹ inhibition was occasionally 100% (Barrett, unpublished). However, the long-term effects still need to be determined, especially on annual species. Flowering tended to be less affected than fruiting and often abortions of immature fruit were observed. In a number of species seed germination was markedly reduced by phosphite treatment and this reduction varied between species. Seed germination in some species was adversely affected by all phosphite rates, whilst in others it was only affected at 144 kg phosphite ha⁻¹ (Barrett, unpublished). Phytotoxicity ratings did not in general correlate directly with effects on flowering and fruiting. In some species phytotoxicity ratings were low, yet phosphite had a marked effect on fruiting.

Stimulation of biochemical defence mechanisms

Although phosphite has been shown to be effective in the control of *P. cinnamomi* in *E. marginata* (jarrah), the biochemical mechanisms behind phosphite protection are poorly understood. Using an aeroponics system (Burgess et al, 1998) jarrah clones resistant to *P. cinnamomi* were treated with foliar applications of phosphite (0 and 5 g L⁻¹). The roots were then inoculated with zoospores of *P. cinnamomi* at 4 days before and 0, 2, 5, 8 and 14 days after phosphite application. Root segments were then analysed for the activity of selected host defence enzymes (4-coumarate coenzyme A ligase [4-CI], cinnamyl alcohol dehydrogenase [CAD] and the concentration of soluble phenolics and phosphite. Lesions were most effectively reduced when the phosphite concentrations were the highest within roots between 8-14 days. During this time, the levels of host defence enzymes remained relatively unchanged. Lesions were also effectively restricted when phosphite concentrations within the roots were lowest (between days 2 and 5). However, a significant increase in host defence enzymes was associated with this decrease in lesion development in the absence of high phosphite tissue concentrations. We concluded that the control of the pathogen by phosphite is determined by phosphite concentration at the host-pathogen interface. When phosphite concentrations within the roots are low, phosphite interacts with the pathogen at the site of ingress to stimulate host defence enzymes. Whilst at high phosphite concentrations, phosphite acts directly on the pathogen to inhibit its growth before it is able to establish an association with the host. At this time, host defences remain unchanged (Jackson et al, 2000). There is still a need to examine how phosphite stimulates plant defences at the biochemical level.

Timing of phosphite application

Timing of phosphite application does not appear to influence the effectiveness of disease control. We found similar results between plants sprayed in spring and autumn. Generally, phosphite is sprayed in autumn in Western Australia since most plants are not flowering at this time, which should reduce any detrimental effects to reproduction at this time. However, we did find that if plants were drought-stressed, uptake of phosphite was less effective than in non-stressed plants. Therefore, it is appropriate to apply phosphite when the plant is not dormant or drought stressed as the chemical is not taken up effectively under these conditions.

Differences between glasshouse and 'field' trials

There are large differences in the uptake of phosphite and phytotoxicity in plants grown in the glasshouse and those growing naturally in the wild. For example, after spray application of phosphite the concentrations of phosphite in glasshouse grown plants are much higher than those recorded in plants growing in the wild. Wilkinson et al (2000c) found that jarrah grown in the glasshouse and treated with phosphite contained 8 times more phosphite than jarrah

in a rehabilitated minesite. The higher rates could be due to the higher relative humidity in the glasshouse compared to the rehabilitated mine sites. 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. (Fairbanks et al. (2000) found the levels of phosphite in glasshouse grown *Eucalyptus calophylla* 7 days after a 10gL^{-1} phosphite treatment to run-off to reach 3561 and $2550\ \mu\text{g g}^{-1}$ in root tips and shoot tips, respectively. Also in the glasshouse, plants appear not to be so sensitive to the phytotoxic effects of phosphite as they are in the field.

Phosphite resistant *Phytophthora cinnamomi* isolates

Recently we have found that there is evidence of *P. cinnamomi* resistance to phosphite treated plants among isolates from native vegetation which have not been exposed previously to phosphite (Wilkinson et al. 1999b; Hüberli et al. 2000). This observation is of concern especially since large areas of vegetation in Western Australia are being and will be sprayed at regular intervals in the future. Regular spraying will provide a selection pressure for these more phosphite resistant isolates and could pose additional problems to managers in the future. There is also some evidence that these more phosphite 'tolerant' isolates are more pathogenic than the less 'tolerant' isolates. Therefore, more research is required to examine these observations in detail. In addition, there is not a good correlation between phosphite tolerance *in vitro* and that *in planta*. Seventy-one isolates of *P. cinnamomi* (68 from Western Australia) were tested for sensitivity to phosphite on agar. Isolates could be divided into sensitive (9% of isolates), intermediate (82% of isolates) and tolerant (9% of isolates) groups. Sensitivity varied greatly between isolates with EC50 values ranging from 4 to $148\ \mu\text{g phosphite/mL}$ (Wilkinson et al. 2000a). Selected isolates that were tolerant to phosphite *in vitro* were not tolerant to phosphite *in planta* (Wilkinson et al. 1999; Wilkinson et al. 2000c). Therefore, in order to screen for phosphite tolerant isolates of *P. cinnamomi* it appears to be a requirement that screening is conducted *in planta*. In addition, *P. cinnamomi* can be isolated from plants that have effectively stopped its colonisation and walled it off and it can be isolated from months to years after being contained (Ali and Guest, 1989; Shearer, pers. com.; Pilbeam, 2000b). The levels of phosphite *in planta* are often higher than those in agar on which the isolate would be inhibited. For example, Wilkinson et al. (2000c) found phosphite concentrations detected in stems of *Banksia hookeriana* and *E. marginata* in a glasshouse trial to be 40 and 14 times higher, respectively, than the highest levels used *in vitro*. However, the growth inhibition of the isolates *in planta* were less than those *in vitro*. Therefore, 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 used in this study would have been more inhibited *in planta* than *in vitro*, and this was not the case (Wilkinson et al. (2000c). One isolate was found to be less inhibited than 5 other isolates when inoculated into phosphite treated jarrah in the field. This isolate was also the most tolerant of the 5 isolates to phosphite *in vitro*. However, another isolate found to be tolerant to phosphite *in vitro* was the most inhibited in plants on the minesite.

In conclusion, there appears to be no correlation between phosphite sensitivity *in vitro* and *in planta*.

THE FUTURE

It will be necessary to determine why the effectiveness of foliar applications of phosphite appear to be less effective than trunk injections. Shearer and Fairman (1997) have found that trunk injections can effectively contain the pathogen for longer than 5 years. In contrast, our results indicate that approximately 6 months after foliar application, the affects of phosphite are disappearing in some plant communities and that these applications will need to be repeated approximately every 2 years.

It will be beneficial to screen more surfactants and sticking agents at different concentrations with phosphite to see if phosphite uptake into plants can be increased without causing increased phytotoxicity.

More work needs to be conducted on the biochemistry of how phosphite activates plant defence mechanisms and these should be compared between *P. cinnamomi* tolerant and susceptible species. For example, in *E. marginata* (jarrah) the activation of defence mechanisms is much less pronounced than in *B. grandis* after phosphite application (Shearer, pers. comm.). An understanding of how phosphite stimulates plant defence mechanisms may allow us to improve these mechanisms through other means.

The continued poor reproductive performance 12–17 months post-phosphite treatment is of some concern. This is exacerbated by our observation that in order to control *P. cinnamomi* it is likely to be necessary to apply phosphite as a foliar application at least every 2 years. Therefore, it will be necessary to determine if regular spraying will exacerbate the effects on reproduction and how this will influence seed banks of perennial and annual species. The question of seed bank viability is important, since regular burns of the natural plant communities are made approximately every 7-10 years. However, it could be argued that *P. cinnamomi* is having a detrimental on plant communities where it is present and without phosphite these species (especially rare and endangered species) will be lost permanently. At least with phosphite germplasm material can be maintained for the future. Despite this we need to better understand the long-term impacts on plant reproduction with the continued use of phosphite.

It will be beneficial to examine host-environment-pathogen interactions in more detail after phosphite treatment, with particular emphasis on its uptake, persistence and effectiveness. There is some evidence that temperature, plant water status and nutritional status all influence the effectiveness of controlling *P. cinnamomi* in its hosts.

In conclusion, phosphite provides us with a very effective and cheap method of reducing the impact of diseases caused by *P. cinnamomi* in natural plant communities and rehabilitated minesites, and currently, it is really the only tool we have. It is, however, important not to rely on this chemical indefinitely and to continue research activities on finding other control strategies for this devastating plant pathogen.

ACKNOWLEDGEMENTS

I acknowledge the contributions made by the studies of Sarah Barrett, Janet Box, Meredith Fairbanks, Kay Howard, Daniel Hüberli, Ros Pilbeam, Kim Tynan, and Carla Wilkinson. Many thanks to Ian Colquhoun and Alcoa World Alumina, Bryan Shearer and Inez Tommerup for their continued support and collaboration.

REFERENCES

- Burgess, T., McComb, J., Hardy, G. (1998). The use of aeroponic chambers to study the influence of low oxygen levels on root and lesion development of *Eucalyptus marginata* Donn. Ex Smith infected with *Phytophthora cinnamomi* Rands. *Plant Disease* **82**, 368-373.
- Colquhoun, I. J. and Hardy, G. E. St J. (2000). Managing the risks of *Phytophthora* root and collar rot during bauxite mining in the *Eucalyptus marginata* (jarrah) forest of Western Australia *Plant Disease* **84**, 116-127.
- Colquhoun, I. J. (2000). This publication.
- Fairbanks M. M., Hardy G. E. St. J., and McComb J. A. (1998). The effect of the fungicide phosphite on pollen germination, pollen fertility and seed germination. In: Proceedings of the XVth International Congress on Sexual Plant Reproduction, Wageningen (Abstract).
- Fairbanks, M. M., Hardy, G. E. St. J., and McComb, J. A. (1999). Does phosphite affect pollination and seed viability in native plants? Abstract 57. Proceedings of the Ecological Society of Australia, Inc. Annual Conference, Fremantle, Western Australia.

- Farih, A., Tsao, P., and Menge, J.** (1981). Fungitoxic activity of fosite aluminium on growth, sporulation and germination of *Phytophthora parasitica* and *P. citrophthora*. *Phytopathology* 71:934-936.
- Guest, D. I., and Bompeix, G.** (1984). Fosetyl-Al as a tool in understanding the resistant response in plants. *Phytophthora Newsletter* 12, 62-69.
- Guest, D. I., and Bompeix, G.** (1990). The complex mode of action of phosphonates. *Aust. Plant Path.* 19, 113-115.
- Guest, D., and Grant, B.** 1991. The complex action of phosphonates as antifungal agents. *Biol. Rev.* 66, 159-187.
- Hardy, G. E. St. J.** (2000). Managing the risks of *Phytophthora* root and collar rot during bauxite mining in the *Eucalyptus marginata* (jarrah) forest of Western Australia This publication.
- Jabahi-Hare, S. H. and Kendrick, W. B.** (1987). Response of an endomycorrhizal fungus in *Allium porrum* L. to different concentrations of the systemic fungicides metalaxyl (Ridomil) and fosetyl-Al (Aliette). *Soil Biology and Biochemistry* 19, 95-99.
- Jackson, T. J., Burgess, T., Colquhoun, I. J., and Hardy G. E. St. J.** (2000). Biochemical effects of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. *Plant Pathology* 49, 147-154.
- Komorek, B. M., Shearer, B. L. Smith, B. and Fairman, R.** (1997). The control of *Phytophthora* in native plant communities. Final Report to the Threatened Species and Communities Unit, Environment Australia. Department of Conservation and Land Management, Como, Western Australia.
- Komorek, B. M., and Shearer, B. L.** (1997). Phosphonate offers a practical method for the control of *Phytophthora cinnamomi* in native plant communities. In: Proceedings of the Australasian Plant Pathology Society Eleventh National Conference, Perth, Western Australia (Abstract).
- Ouimette, D. G., and Coffey, M. D.** (1989). Comparative antifungal activity of four phosphonate compounds against isolates of nine *Phytophthora* species. *Phytopathology* 79, 761-767.
- Ouimette, D. G., and Coffey, M. D.** (1990). Symplastic entry and phloem translocation of phosphonate. *Pesticide Biochemistry and Physiology* 38, 18-25.
- Pilbeam, R. A., Colquhoun, I. J., Shearer, B., and Hardy, G. E. St. J.** (2000). Phosphite concentration: Its effect on phytotoxicity symptoms and colonisation by *Phytophthora cinnamomi* in three understorey species of *Eucalyptus marginata* forest. *Aust. Plant Path.* (in press).
- Pilbeam, R. A., Colquhoun, I. J., Shearer, B., and Hardy, G. E. St. J.** (2000). The extension of *Phytophthora cinnamomi* in *Eucalyptus marginata* stems inoculated at different times after treatment with three concentrations of phosphite. *Australasian Plant Pathology* (in preparation).
- Howard, K., Dell, B., and Hardy, G. E. St. J.** (1999). Phosphite has no effect on ectomycorrhizal formation or persistence. Abstract 322 in: Proceedings of the Australasian Plant Pathology Society Twelfth National Conference, Canberra, Australian Capital Territory, Australia.
- Howard, K., Dell, B., and Hardy, G.** (2000). Phosphite and mycorrhizal formation in seedlings of three Australia Myrtaceae. *Australian Journal of Botany* (in press).
- Hüberli, D., Tommerup, I. C. and Hardy, G. E. St. J.** (2000b). How variable are morphological and pathological phenotypes among *Phytophthora cinnamomi* isolates from two localised populations in Western Australia (to be submitted to *Mycological Research*).
- Roos, G. H. P., Loane, C., Dell, B., and Hardy, G. E. St. J.** (1999). Facile high performance ion chromatographic analysis of phosphite and phosphate in plant samples. *Communications in Soil Science and Plant Analysis*. 30, 2323-2329.
- Saindrenan, P., Barchietto, T., Avelino, J. and Bompeix, G.** (1988). Effects of phosphite on phytoalexin accumulation in leaves of cowpea infected with *Phytophthora cryptogea*. *Physiological and Molecular Plant Pathology* 32, 425-35.
- Seymour, N. P., Thompson, J. P., and Fiske, M. L.** (1994). Phytotoxicity of fosetyl-Al and phosphonic acid to maize during production of vesicular-arbuscular mycorrhizal inoculum. *Plant Disease* 78, 441-446.
- Shearer B. L., and Fairman R. G.** (1997). Foliar application of phosphite delays and reduces the rate of mortality of three *Banksia* species in communities infested with *Phytophthora cinnamomi*. In: Proceedings of the Australasian Plant Pathology Society Eleventh National Conference, Perth, Western Australia (Abstract).
- Smillie, R. H. , Grant, B. R. and Guest, D.** (1989). The mode of action of phosphite: evidence for both direct and indirect modes of action on three *Phytophthora* spp. in plants. *Phytopathology* 79, 921-926.
- Sukarno, N., Smith, F. A., Smith, S. E. and Scott, E. S.** (1996). The effects of fungicides on vesicular-arbuscular mycorrhizal symbiosis. II. The effects on area of interface and efficiency of P uptake and transfer to plant. *New Phytologist* 132, 583-592.
- Sukarno, N., Simth, F. A., Scott, E. S., Jones, G. P., and Smith, S. E.** (1998). The effects of fungicides on vesicular-arbuscular mycorrhizal symbiosis. III. The influence of VA mycorrhiza on phytotoxic effects following application of fosetyl-Al and phosphonate. *New Phytologist* 139, 321-330.
- Tommerup, I. C., Dobrowolski, M.P., Hüberli, D., O'Brien, P.A., Hardy, G. E. St. J. and Shearer, B.L.** (2000). Risks and threats due to genetic variation in *Phytophthora cinnamomi* for disease management in natural vegetation ecosystems. This publication.
- Wiley, A. W., Hargreaves, P. A., Pegg, K. G., Doogan, V. J. Ruddle, L. J., Saranah, J. B. and Langdon, P. W.** (1995). Changing sink strengths influence translocation of phosphonate in avocado (*Persea americana* Mill.) tree. *Australian Journal of Agricultural Research* 46, 1079-1090.
- Wilkinson, C., Shearer, B., Holmes, J., Tynan, K., Dell, B., McComb, J., Maroudas, J., and Hardy, G. E. St. J.** (1999). A comparison of the *in vitro* and *in planta* responses of *Phytophthora cinnamomi* isolates to phosphite. Abstract 231 in: Proceedings of the Australasian Plant Pathology Society Twelfth National Conference, Canberra, Australian Capital Territory, Australia.
- Wilkinson, C., Hardy, G. E. St. J., and Shearer, B. L.** (1997) The effect of phosphite (phosphonate) on *Phytophthora cinnamomi* zoospore production *in planta*. In: Proceedings of the Australasian Plant Pathology Society Eleventh National Conference, Perth, Western Australia (Abstract).
- Wilkinson, C., Shearer, B., Holmes, J., Tynan, K., Dell, B., McComb, J., Maroudas, J., and Hardy, G. E. St. J.** (1999). Does phosphite prevent zoospore production from *Phytophthora cinnamomi*-colonised plants in the field? Abstract 230 in: Proceedings of the Australasian Plant Pathology Society Twelfth National Conference, Canberra, Australian Capital Territory, Australia.
- Wilkinson, C, Shearer, B. L., Jackson, T. J. and Hardy, G. E. St. J.** (2000a). Variation in sensitivity of Western Australian isolates of *Phytophthora cinnamomi* to phosphite *in vitro*. *Australasian Plant Pathology* (in press).
- Wilkinson, C., Shearer, B., Holmes, J., Colquhoun, I. , Tynan, Dell, B., and Hardy, G. E. St. J.** (2000b). The effect of phosphite on *Phytophthora cinnamomi* zoospore production *in planta*. *Plant Disease* (submitted).
- Wilkinson, C. J. , Holmes, J. M., Dell, B., Tynan, K. M., McComb, J. A., Colquhoun, I. J., and Hardy, G. E. St. J.** (2000c). Sensitivity of *Phytophthora cinnamomi* isolates to phosphite *in planta*. *Plant Disease* (submitted).