
http://researchrepository.murdoch.edu.au/2536/

Copyright: © 2000 CSIRO

It is posted here for your personal use. No further distribution is permitted.
Phosphite and mycorrhizal formation in seedlings of three Australian Myrtaceae

Kay Howard, Bernie Dell and Giles E. Hardy

School of Biological Science and Biotechnology, Murdoch University

Abstract

Currently in Western Australia, phosphite is being used to contain the root and collar rot pathogen, Phytophthora cinnamomi, in native plant communities. There have been reports of negative effects of phosphite on arbuscular mycorrhiza (AM), so there are concerns that it may have a deleterious effect on other mycorrhizal fungi. Two glasshouse experiments were undertaken to determine the impact of phosphite on eucalypt-associated ectomycorrhizal fungi. In the first experiment, non-mycorrhizal seedlings of Eucalyptus marginata, Eucalyptus globulus and Agonis flexuosa were sprayed to runoff with several concentrations of phosphite, and then planted into soil naturally infested with early colonising mycorrhizal species. Assessments were made of percentage of roots infected with mycorrhizal fungi. There was no significant effect on ectomycorrhizal formation but there was a four-fold increase in AM colonisation of A. flexuosa roots with phosphite application. In the second experiment, E. globulus seedlings mycorrhizal with Pisolithus, Scleroderma and Descolea were treated with different levels of phosphite and infection of new roots by ectomycorrhizal fungi was assessed. There was no significant effect on ectomycorrhizal formation when phosphite was applied at the recommended rate (5 g L−1), while at 10 g L−1 phosphite significantly decreased infection by Descolea.
Introduction

Phosphite is a systemic fungicide that can be applied as a drench, foliar spray or by trunk injection to control a number of Oomycete diseases in many horticultural and ornamental crops (Dolan and Coffey 1988; Pegg et al. 1990; Wicks and Hall 1990; Greenhalgh et al. 1994; El-Hamalawi and Menge 1995). In Western Australia, *Phytophthora cinnamomi* Rands is an aggressive root pathogen that causes dieback in a wide range of native plant species (Shearer 1994; Shearer and Dillon 1995). The Department of Conservation and Land Management (CALM) in Western Australia has been trialing aerial applications of phosphite to protect small areas of native bushland from *P. cinnamomi* (Komorek and Shearer 1997). In addition, researchers have been successful in using phosphite to protect threatened native flora (Pilbeam et al. 2000).

Phosphite may act directly upon the invading fungus within the plant tissue causing inhibition or death, or indirectly by activating plant defence responses (Grant et al. 1990). Although many studies and reviews on the effect of phosphite on *Phytophthora* have been published (Smillie et al. 1989; Nemestothy and Guest 1990; Guest and Grant 1991), few workers have investigated the effects of phosphite on beneficial fungi associated with plants. Ectomycorrhizal (ECM) fungi are important components of the soil biota, especially on nutrient poor soils, and have potential to be economically beneficial in commercial forestry (Bougher 1994). Arbuscular mycorrhizal (AM) fungi are present in over two thirds of vascular plant species (Newman and Reddell 1987). They are obligate symbionts, indigenous to soils throughout the world, and are not host specific (Daniels Hetrick 1984; Powell and Bagyaraj 1984). Experiments on the effect of phosphite application on monocotyledonous
plants with AM are contradictory. For example, Jabahi-Hare and Kendrick (1987) observed an increase in AM in phosphite-treated leek, whereas phosphite decreased AM in maize (Seymour et al. 1994) and onion (Sukarno et al. 1996, 1998).

Phosphite, transported via the phloem, accumulates near root tips in the area that is colonised by mycorrhizal fungi (Guest and Grant 1991). Where phosphite accumulates it can cause necrosis. In plant species that form symbiotic relationships with mycorrhizal fungi, phosphite damage to fine roots may cause a reduction in sites for ECM and AM formation. As mycorrhizal fungi increase the longevity of fine roots, and active fine roots act as a metabolic sink for photosynthates (and therefore phosphite) the application of this fungicide may damage the mycorrhizal interface. In addition, any damage to young roots will alter the pattern of root exudates, and may cause a change in the soil microflora (Wongwathanarat and Sivasithamparam 1991) especially in P-deficient soils, thus indirectly affecting mycorrhizae. Changes in exudates, for example, could adversely affect the role of mycorrhiza-helper bacteria (Dunstan et al. 1998) at the interface and may cause competition between mycorrhizal species in the natural environment, resulting in a change of mycorrhizal infection and/or succession.

The sensitivity of ECM species to phosphite has not been investigated. In Western Australia, myrtaceous species, which are predominantly ectomycorrhizal (Bougher 1994), are a common constituent of the vegetation being degraded by *P. cinnamomi*. We report here on the effects of phosphite on phytotoxicity, plant growth and the formation of ECM by two tree species native to the Western Australian jarrah forest, *Eucalyptus marginata* Donn. ex Smith (jarrah) and *Agonis flexuosa* Willd. (native peppermint), and a species from eastern Australia,
Eucalyptus globulus Labill. (bluegum), which is widely used as a plantation species throughout southern Australia.

**Methods**

*Experiment 1: mycorrhizal formation from soil inoculum*

A glasshouse experiment was conducted to test the effect of phosphite on the ability of plants to form mycorrhizae in a soil naturally infested with ECM fungi. Fifty *E. marginata* and *E. globulus* seedlings were treated with five phosphite concentrations and 40 *A. flexuosa* seedlings were treated with four phosphite concentrations. Plant height, root and shoot dry weight, and the proportion of the root that was mycorrhizal were measured.

**Soil**

A greyish sandy loam (2.1 ± 0.06% organic carbon; Rayment and Higginson 1992a) was collected (0–20 cm) from a 5-year-old *E. globulus* plantation (McDonalds Tree Farm, near Augusta, Western Australia). ECM fungi observed to fruit at this site are *Amanita* (one species), *Descolea* (one species), *Laccaria* (one species) *Pisolithus* (one species) and *Scleroderma* (three species) (Lu et al. 1998). The available-P content of the soil was 19 ± 3.9 mg kg⁻¹ and the pH was 4.4 ± 0.06 (Rayment and Higginson 1992b). About 1 kg of sieved (1-cm² mesh) and mixed soil was added to 130-mm-diam. free-draining pots (Pola Cup, Western Australia).
Phosphite treatments

Seedlings were germinated in 48 cell trays (Smith and Nephew, Western Australia) containing steam sterilised peat/perlite (2:1, v:v). When plants were 2 months old they were removed from the cells and the root balls were covered with non-absorbent cotton wool to prevent roots coming in direct contact with the phosphite. No ectomycorrhizae were observed on the outside of the root ball. Foliage of *E. marginata* and *E. globulus* seedlings was sprayed to runoff with 0, 2.5, 5, 7.5 and 10 g phosphite L⁻¹ (Fosject 400, UIM Agrochemicals), and 10-month-old *A. flexuosa* seedlings were sprayed with 0, 5, 7.5 and 10 g phosphite L⁻¹. The phosphite solution contained 0.25% of the sticking agent Synertrol (Organic Crop Protectants, NSW) and was applied with a handheld pump-action spray bottle. The rate recommended for the control of *P. cinnamomi* in Western Australian native plants is 5 g phosphite L⁻¹ (C Wilkinson, pers. comm.). After spraying, the plants were immediately planted (one plant/pot) and the soil was hand watered for the first 48 h to avoid wetting the foliage. There were 10 replicates for each treatment.

Plant growth and phytotoxicity

The plants were kept in a heated glasshouse (temperature range 15–30°C), with overhead watering twice a day. No fertilisers or fungicides were applied for the duration of the experiment. Plant height was recorded at 0, 30 and 90 days after spraying. Phytotoxicity was assessed at 14 days by rating the extent of leaf burn as follows; 0 = no visible effect, 1 = slight (leaf tips chlorotic/burnt, margins chlorotic), 2 = moderate (growing shoot tip burnt, older leaves chlorotic), 3 = severe (growing tip burnt, more than ½ of each leaf burnt/chlorotic, leaf drop).
**Harvest**

After 90 days, the shoots were severed at the lignotuber (*E. marginata*) or at the shoot/root junction (*E. globulus* and *A. flexuosa*) and the roots were washed over a 210-μm sieve. A subsample (1 g fresh weight) of fine roots was fixed in 50% ethanol for microscopic study. The remainder of the roots and the whole shoot were dried at 60°C to constant weight and weighed.

**Mycorrhizal assessment**

A subsample (0.2 g) of roots was cleared by autoclaving (10% KOH) for 20 min, rinsed in water, stained in lactoglycerol trypan blue for 4 h, then destained in lactoglycerol (Brundrett et al. 1996). ECM were quantified by the intercept method as described in Brundrett et al. (1996). Root tips infected with *Laccaria* were identified by their smooth dense mantle, while *Pisolithus* and *Scleroderma* were recognised as a dense fungal sheath and thickened root tips. The total length of root infected with vesicles and arbuscules (AM) was also calculated.

**Experiment 2: spread of inoculum from old to young roots**

A second experiment was conducted to determine whether phosphite affected the ability of ectomycorrhiza from established ECM plants to colonise new roots. A total of 120 1-year-old *E. globulus* seedlings were treated with three concentrations of phosphite.
*Eucalyptus globulus* seeds were inoculated with solutions of 106, 106 and 107 spores/mL of *Laccaria*, *Descolea* and *Pisolithus* species, respectively (Lu *et al*. 1998), at the time of sowing into 64 cell trays containing steam-sterilised peat/perlite (2 : 1, v : v). Control seedlings were not inoculated. Phosphite (0, 5 and 10 g L\(^{-1}\)) was applied as in the first experiment, and the seedlings were planted into steam-sterilised peat/perlite (2 : 1, v : v), pH 6.0, in free-draining 75 × 75 × 100-mm squat pots (Pacific Pots, Western Australia). There was one plant per pot and 10 replicates for each treatment. The plants were kept in a heated glasshouse (19–32°C). After 90 days, new roots were sampled by taking two cores (10 mm diam. × 40 mm) of the soil 1 cm from the edge of the original root ball of each plant. Mycorrhizae were determined as described earlier.

**Results**

**Experiment 1**

*Phytotoxicity and plant growth*

Symptoms of phytotoxicity appeared on all plants within 48 h of phosphite application. Margins and tips of young leaves and the meristematic regions were necrotic by 72 h. The margins and tips of older leaves became chlorotic within 48 h. No new symptoms were detected after 14 days and subsequent new growth of all plants was normal. Symptoms increased in all species as phosphite concentration increased (Fig. 1). *Eucalyptus marginata* was most affected, with moderate phytotoxicity at 7.5 and 10 g phosphite L\(^{-1}\), where most of the growing shoot tips were necrotic and the older leaves had some chlorosis. *Eucalyptus globulus* was less affected than *E. marginata* at 5 g (*P* = 0.006) and 7.5 g phosphite L\(^{-1}\) (*P* =
0.005), and appeared more chlorotic than burnt. The growing shoot tip was more susceptible in *A. flexuosa* than in the eucalypts, and leaf drop occurred at 10 g phosphite L$^{-1}$ in *A. flexuosa*.

Phosphite decreased the height (*P* = 0.001) of *A. flexuosa* at 30 and 90 days at all concentrations, by 24–30% and 12–20%, respectively. Phosphite also decreased the shoot dry weight (*P* = 0.02) of *A. flexuosa* by 12–34% compared with non-treated plants. In contrast,

![Phytotoxicity ratings](image)

**Fig. 1.** Phytotoxicity ratings 14 days after application of phosphite to *Eucalyptus marginata* (O) *E. globulus* (□) and *Agonis flexuosa* (♦). Values are means of 10 observations with one s.e. bar shown. Phytotoxicity rating from 0 (no visible effect) to 3 (severe symptoms).
phosphite did not affect plant height or shoot dry weight in *E. marginata* and *E. globulus* (*P* = 0.91 and 0.09, respectively). The dry weight of roots was not affected (*P* = 0.1) by phosphite treatment in any of the three species.

**Mycorrhiza**

Phosphite did not (*P* > 0.4) affect the proportion of roots colonised by ECM fungi from natural soil inoculum (Table 1). The average percentage of roots colonised by ECM fungi for each species was *E. marginata* 24 ± 0.9, *E. globulus* 27 ± 0.5 and *A. flexuosa* 20 ± 1.6%.

Three main types of mycorrhiza were observed: those with long, smooth, dense mantles; those with dense fungal mantles on thick, short lateral roots; and those with sparse sheaths of thin hyphae on thickened root tips.

**Table 1. Percentage of roots colonised by ectomycorrhizal fungi 90 days after phosphite application in Eucalyptus marginata, E. globulus and Agonis flexuosa (P-values from ANOVA are 0.79, 0.89 and 0.40, respectively)**

Values are means of 10 observations; n.t., not tested

<table>
<thead>
<tr>
<th>Phosphate concentration (g L⁻¹)</th>
<th>Eucalyptus marginata</th>
<th>Eucalyptus globulus</th>
<th>Agonis flexuosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>26 ± 3</td>
<td>27 ± 2</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>2.5</td>
<td>24 ± 4</td>
<td>27 ± 2</td>
<td>n.t.</td>
</tr>
<tr>
<td>5</td>
<td>25 ± 3</td>
<td>29 ± 2</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>7.5</td>
<td>26 ± 2</td>
<td>27 ± 2</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>21 ± 2</td>
<td>26 ± 2</td>
<td>16 ± 2</td>
</tr>
</tbody>
</table>
While the two eucalypt species tested had less than 1% AM present in all treatments, the *A. flexuosa* roots had a fourfold increase in AM colonisation (Table 2) after phosphite treatment. The arbuscules observed were the Arum type as described by Smith and Smith (1997).

**Experiment 2**

Before phosphite treatment, the percentage of mycorrhizal roots was *Laccaria* 21.4 ± 0.8, *Descolea* 32.6 ± 1.3 and *Pisolithus* 18.2 ± 1.0%. In these synthesised mycorrhizal associations, phosphite did not affect the percentage of new mycorrhizal roots in plants inoculated with *Laccaria* (*P* = 0.44) and *Pisolithus* (*P* = 0.26). Treatment with 10 g phosphite

<table>
<thead>
<tr>
<th>Phosphite concentration (g L⁻¹)</th>
<th>Roots colonised (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8 ± 3a</td>
</tr>
<tr>
<td>5</td>
<td>32 ± 5b</td>
</tr>
<tr>
<td>7.5</td>
<td>32 ± 6b</td>
</tr>
<tr>
<td>10</td>
<td>27 ± 5b</td>
</tr>
</tbody>
</table>
Fig. 2. Percentage of new roots colonised by ectomycorrhizal fungi in *Eucalyptus globulus* 90 days after treatment with 0 (□), 5 (□) and 10 g phosphite L⁻¹ (□). The *Thelephora* results are for the uninoculated plants. Values are means of 10 observations with one s.e.

L⁻¹ (*P* = 0.026) decreased the percentage of roots infected with *Descolea* in comparison with the non-treated control (Fig. 2). About 10–15% of the roots of the control plants were contaminated with a sparse sheath of white fungal hyphae at the root tips. This was attributed to *Thelephora* whose fruiting bodies were frequently observed. Phosphite did not affect (*P* = 0.94) the incidence of this fungus in the uninoculated plants. Similar low levels of contamination occurred in the seedlings inoculated with *Laccaria, Pisolithus* and *Descolea*, but the data are excluded from Fig. 2.
Discussion

At the recommended rate of 5 g L\(^{-1}\), the application of phosphite to three Australian tree species, at the seedling stage, did not significantly affect formation of ectomycorrhiza (Experiment 1), nor the colonisation of new roots in *E. globulus* inoculated with three species of ECM fungi (Experiment 2). However, infection of new roots by *Descolea* was significantly decreased in plants that had been sprayed with 10 g of phosphite per litre.

Once phosphite enters the plant it is translocated throughout the plant and accumulates at actively growing sites (Whiley *et al.* 1995). As in phosphate toxicity, too much phosphite results in phytotoxic symptoms in the leaves. In a related glasshouse study, there was no correlation between phytotoxic symptoms in the shoot and the root of *Banksia brownii* treated with phosphite (S Barrett, pers. comm.). In the current study, no phytotoxic symptoms were seen in the root tips of the three species. However, brown discolouration of the stele occurred in fine roots of *B. brownii* treated with 96 kg phosphite per hectare (S Barrett, pers. comm.) and browning of root tips was observed in aeroponically grown *E. marginata* treated with 5, 10 and 15 g phosphite per litre (Jackson *et al.* 2000).

The increased colonisation of AM in phosphite-treated *A. flexuosa* was similar to the increase in AM observed in phosphite-treated leek (Jabahi-Hare and Kendrick 1987). This increase may be attributed to differences in nutritional uptake from the host or altered host response. With the fungicide Fosetyl-Al (active ingredient phosphite) Jabaji-Hare and Kendrick (1987) reported a significant increase in soluble sugars in root exudates, which also occurs when
phosphate is limiting. A change in the carbohydrate and amino acid flow from roots into the rhizosphere may influence AM and ECM spore germination. Therefore, the effect of altered root metabolism in phosphite-treated plants needs further investigation.

It is also possible that phosphite may disrupt some events in the cascade of gene expression that results in host/fungus recognition and the development of a functional symbiosis. For example, even though new roots continued to form in *E. globulus* inoculated with *Descolea*, there was reduced ectomycorrhizal development. Saindrenan *et al.* (1990) showed that elicitor activity was enhanced by phosphite in *Phytophthora cryptogea* and it caused host cell necrosis and subsequently less infection.

This is the first study to show that phosphite applied as a foliar spray, at the recommended level of 5 g phosphite per litre, does not significantly affect the development of ectomycorrhizae on roots. However, there is still a need to examine the formation of the mycorrhizal mantle and Hartig net, and it is essential that longer-term studies are undertaken, especially if plants are regularly treated with phosphite. Further research should include a larger number of ECM fungi, including late-colonising fungi, more isolates of ECM fungi and more hosts. It is also necessary to determine whether phosphite has any effect on the germination of spores of mycorrhizal fungi and their ability to infect roots, and whether there is any effect on the fruiting capacity of mycorrhizal fungi. Sporocarps are important for sexual reproduction of the fungus and in the eucalypt forest many hypogeous fungi provide a food source for forest dwelling animals that facilitate spore dispersal. Thus, it is important to show that phosphite applied to natural vegetation on a regular basis does not affect these fungi.
Acknowledgements

The senior author was financially supported by an APAscholarship. We gratefully acknowledge Xianheng Lu for supplying plants for Experiment 2, Bunnings Treefarms for allowing access to *E. globulus* plantations, and Jen McComb for reviewing the manuscript.

References


Komorek BM, Shearer BL (1997) Application technologies and phosphonate movement in the host. In ‘Control of *Phytophthora* and *Diplodina* canker in Western Australia’. pp. 1–59. (Conservation and Land Management: Bentley, WA)


