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# Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*

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Although phosphite has been effective in the control of *P. cinnamomi* in *E. marginata* (jarrah), the biochemical mechanisms behind phosphite protection are poorly understood. Using an aeroponics system, jarrah clones with moderate resistance to *P. cinnamomi* were treated with foliar applications of phosphite (0 and 5 g $L^{-1}$). The roots were inoculated with zoospores of *P. cinnamomi* at 4 days before and 0, 2, 5, 8 and 14 days after phosphite treatment. Root segments were then analysed for activity of selected host defence enzymes (4-coumarate coenzyme A ligase [4-CL], cinnamyl alcohol dehydrogenase [CAD]) and the concentration of soluble phenolics and phosphite. Lesion development was most effectively reduced when phosphite concentrations within the roots were highest (i.e. days 8–14). During this time, the levels of host defence enzymes remained relatively unchanged. Lesion development was also effectively restricted when phosphite concentrations within the roots were lowest (i.e. days 2 and 5); a significant increase in host defence enzymes was associated with this decrease in lesion development. It was concluded from these studies that the effect of phosphite in controlling the pathogen is determined by the 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. 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, and the host defences remain unchanged.

*Keywords*: host defence, jarrah, phenolics, phosphonate, *Phytophthora*

## Introduction

*Phytophthora cinnamomi* is an introduced soilborne pathogen affecting the ecology and management of the *Eucalyptus marginata* (jarrah) forests in south-west Western Australia (WA). The pathogen affects over 22% of the plant species in this region (Shearer & Tippett, 1989; Wills, 1992).

Recently, the fungicide phosphite (phosphonate) has been shown to be effective against *P. cinnamomi* in native plant communities in WA (Komorek & Shearer, 1997). Phosphite is a systemic fungicide which is translocated in both the xylem and phloem (Guest & Grant, 1991). After application, phosphite is translocated in the xylem before moving into the phloem. Once inside the phloem, phosphite is trapped and therefore is translocated through the plant in association with photoassimilates in a source–sink relationship (Saindrenan *et al*., 1988; Ouimette & Coffey, 1990; Guest & 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 concentration of phosphite in plant tissues was found to be directly related to the application rate (Smillie *et al*., 1989). Phosphite exhibits a complex mode of action, acting both directly on the pathogen and indirectly in stimulating host defence responses to ultimately inhibit pathogen growth (Guest & Grant, 1991).

It is not known exactly how phosphite is able to induce this resistant state. Accumulation of products of the phenylpropanoid pathway is known to be important in plant defence (Kessman *et al*., 1994). The accumulation of phenolic compounds (an end-product of the phenylpropanoid pathway) is involved in creating a physical and chemical barrier against pathogen invasion (Candela *et al*., 1995). Phenolic compounds have previously been linked with the resistance of *Eucalyptus marginata* to *P. cinnamomi* (Cahill *et al*., 1993). Elicitors and chemicals such as phosphite were found to activate the phenylpropanoid pathway, although phosphite only

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stimulated host defences, including the phenylpropanoid pathway, after pathogen challenge (Saindrenan *et al*., 1988; Nemestothy & Guest, 1990).

Phosphite has been found to be most effective when applied prior to infection, although Marks & Smith (1992) reported a significant reduction in lesion development in *Leucadendron* if the plant was treated with phosphite at the time of *P. cinnamomi* infection. Rohrbach & Schenck (1985) also showed that Fosetyl-Al (phosphonate) was able to protect roots from pathogen invasion 24 h after treatment. Although tissue phosphonate concentrations were not measured in this study, it is suggested that the protection may have been the result of the rapid downward systemic activity of the phosphonate. However, Davis (1989) reported that the effect of phosphite decreased as the time interval between treatment and inoculation increased. Protection from *Phytophthora parasitica* was minimal 3–4 weeks after treatment of tomato plants with Fosetyl-Al, even after multiple applications (Davis, 1989).

Questions still remain concerning the effect of phosphite on the enzymic responses of a host. The critical timing between foliar application of phosphite and effective lesion reduction in roots, via the stimulation of phenylpropanoid enzymes, is still to be determined. Therefore, the current study examined the rate at which foliar applications of phosphite protected roots of *E. marginata* clonal plants challenged with motile zoospores of *P. cinnamomi*.

## Materials and methods

### Aeroponics system

An aeroponics system developed at Murdoch University (Burgess *et al*., 1998) was used to produce roots suitable for inoculation with zoospores of *P. cinnamomi*. Briefly, the aeroponics system consisted of misting chambers (boxes with sides of 60 cm in length) with removable side panels, insulated with polystyrene and insulation foil. The top of each chamber was fitted with 3 removable lids, each with provision to hold 12 plants. Two clear PVC portholes, located on each of the front and back panels of the chambers, allowed observation of and access to the roots within the chamber. The portholes were covered to protect the roots from light. An automatic watering system, installed in and above the chambers, watered the roots and foliage. The roots were watered for 8 s every 20 min, via six evenly spaced misting jets placed at the base of the chambers, whilst the foliage was watered for 1 min twice a day. The aeroponics system was located within an evaporatively cooled glasshouse (20/27°C minimum/maximum).

### Biological material

Clonal *E. marginata* plants (326J51), classified as moderately resistant to infection by *P. cinnamomi* by Stukely & Crane (1994), were supplied by the Alcoa's Marrinup Nursery (PO Box 52, Dwellingup 6213, Australia). The 6-month-old plants were removed from their 10×4-cm plastic pots, their root balls were trimmed and they were placed into open-ended 5×4-cm plastic pots. In order to stimulate root growth out of the root balls, the plants were placed into black plastic boxes of peat/perlite potting mix (2 : 1 v/v) containing basal nutrients (isobutylidene diurea (IBDU) containing 31% nitrogen (0·85 g $L^{-1}$), $KNO_3$ (0·44 g $L^{-1}$), aerophos (0·40 g $L^{-1}$), $FeSO_4$ (0·58 g $L^{-1}$), FeO (1·18 g $L^{-1}$), dolomite (0·78 g $L^{-1}$), gypsum (0·52 g $L^{-1}$) and trace elements (11% K as $K_2SO_4$, 5% Fe as $FeSO_4$, 5% Ca as $CaCO_3$, 2% Mn as $MnSO_4$, 2%Mg as $MgSO_4$, 1·5% Cu as $CuSO_4$, 1% Zn as ZnO, 2% B as $Na_2B_4O_7$ and 1% Mo as $Na_2MoO_4$) (0·010 g $L^{-1}$)). The plants were watered twice daily for 10 min and fertilized by hand twice a week with Phostrogen (5·4 mM N, 0·8 mM P, 4·3 mM K, 0·4 mM Mg, 0·75 mM Ca, 1·1 mM S, 50 $\mu$M Fe, 3 $\mu$M Mn) (Phostrogen Australia Pty Ltd, 12 McKirdys Rd, Tyabb, Victoria, Australia) to stimulate root growth.

Six weeks later, the plants were transferred into the aeroponics chambers. At this stage, the plants were growing vigorously and root development had been initiated. The open-ended plastic pots containing the root balls were suspended in the aeroponics chambers on a plastic mesh which provided support for the developing roots. The plants were grown in the aeroponics system for 6 weeks before the phosphite treatments (at this time, the roots were between 100 and 300 mm long, with lignification commencing approximately 10 cm from the root tip).

### Phosphite application

Phosphite was applied to the plants as a foliar spray to runoff at concentrations of 0 and 5 g $L^{-1}$. The phosphite concentrations were prepared from a 20% w/v Fosject-200 stock solution (UIM Agrochemicals, Aust. Pty. Ltd) containing mono di-potassium phosphonate. Each concentration was mixed thoroughly with 2·5 g $L^{-1}$ Synertrol oil (Organic Crop Protectants Pty Ltd, 36–40 Halloran, St, Lilyfield, NSW 2040). The control treatment (0 g $L^{-1}$) consisted of surfactant and distilled water. The foliage was allowed to dry naturally for 48 h before the automatic watering programme was re-established. The plants were monitored for symptoms of phytotoxicity after spraying.

### Zoospore inoculation

The *P. cinnamomi* isolate MP94–48 used in this study was isolated from a diseased jarrah from Alcoa of Australia's Willowdale rehabilitated minesite. This isolate is highly pathogenic (Hüberli, 1995) and moderately tolerant to phosphite *in vitro* (Wilkinson, 1997). Cultures were maintained on vegetable juice (V8) agar and zoospores were produced according to the method of O'Gara *et al*. (1997). Squares (5 $mm^2$) of actively growing mycelium were transferred onto V8 agar covered by a sterile cheesecloth square. After

incubating the plates in the dark at 24°C for 4 days, the colonized cheesecloth was transferred to an Erlenmeyer flask containing 150 mL of V8 broth. The flasks were shaken overnight at 150 r.p.m. in light (24°C), rinsed thoroughly with mineral salts (5 mM $KNO_3$, 10 mM $Ca(NO_3)_2{\cdot}4H_2O$, 4 mM $MgSO_4{\cdot}7H_2O$) and shaken again overnight in mineral salts to induce sporangium formation. The flasks were then placed on a light box for approximately 2 h, cold-shocked for 20 min and then allowed to return to room temperature (24°C) until the zoospores were released. Zoospores were quantified by determining the mean number present in a 5-$\mu$L aliquot of the zoospore suspension.

In order to inoculate roots, lids were removed from the aeroponics systems in the late afternoon and placed on a stand that allowed access to all roots. The roots were marked 2 cm above the root tip with a vital stain (0·1% methylene blue). A 5-$\mu$L aliquot of zoospore solution, which contained approximately 30 zoospores, was suspended on the root tip using a Gilson pipette. The roots were then allowed to stand for 5 min to enable the zoospores to encyst, before the automatic watering system was resumed. Marking and inoculation of roots took a maximum of 20 min and roots were regularly sprayed with water to prevent desiccation.

The roots were harvested four days after inoculation and the lesion and root extension recorded. The rate of root extension was determined by comparing root growth with the blue stains. Lesions were measured as the water-soaked, discoloured apical regions of the roots. The length of the white root region was also measured. The roots were divided into four segments from the root tip for biochemical analysis. The first segment contained the lesion and the remainder was divided into three segments, each 20 mm in length. Roots showing no visible lesions were surfaced-sterilized with 70% ethanol, cut into 1-cm segments (for 6 cm of the root), plated onto *Phytophthora*-selective medium and incubated in the dark at 24°C to determine whether *P. cinnamomi* was present. The root segments were placed in 1·5-mL Eppendorf tubes, snap frozen in liquid nitrogen and stored at − 80°C.

## Experimental design

Three aeroponic chambers were used, each containing 36 plants. The plants in two of the chambers were treated with phosphite and those in the third with the surfactant/water mixture. Forty-eight hours after phosphite application, the nine lids of the three aeroponics chambers, which held the plants, were completely randomized. The 6-week-old roots were inoculated with 5 $\mu$L of a zoospore suspension 4 days before and 0, 2, 5, 8 and 14 days after phosphite treatment. Roots were harvested 4 days after each inoculation event and lesion development and root extension were measured before the roots were stored as outlined above. Approximately 30–40 roots were inoculated at each inoculation time, with an equal number of noninoculated controls. Noninoculated white roots (approximately 8–10) were collected for phosphite analysis. The experiment was set up in duplicate.

Data obtained from enzymic analysis and root and lesion extension were analysed using Statistica (Statsoft Inc., 2300 East 14th Street, Tulsa, OK 74104).

## Enzyme extraction

The root material was extracted according to Moerschbacher *et al.* (1986), with the following modifications. Briefly, the root material was powdered in a mortar and pestle with liquid nitrogen and 1 mL of extraction buffer (0·1 M sodium borate (pH 8·8) containing 1 mM ethylenediamine tetra-acetic acid (EDTA), 1 mM dithiothreitol (DTT) (added fresh daily), 1 mM ascorbic acid and 1 mM phenylmethylsulfonyl fluoride (PMSF) (added fresh daily)). The extract was then centrifuged in a 1·5-mL Eppendorf tube at 14 000 ***g*** for 10 min at 4°C and a 25-$\mu$L aliquot removed for the phenolic assay. Polyvinylpyrrolidone (PVP) (10% w/v) was added and the extracts were mixed and centrifuged. The soluble extract (supernatant) used for enzyme (4-coumarate CoA ligase, cinnamyl-alcohol dehydrogenase) and protein analysis was transferred into an Eppendorf tube containing 200 $\mu$L of glycerol, mixed thoroughly and stored on ice during the course of the enzyme assays outlined below. The protein content of the supernatant was determined using a Protein Determination Kit (Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547), based on the method of Bradford (1976), and protein expressed as $mg\,mL^{-1}$ by comparing the absorbance at 595 nm with that of a BSA standard curve. The level of soluble phenolics was determined using Folin and Ciocalteu's Phenol reagent (Sigma Chemical Company, Sigma-Aldrich Pty Ltd, Unit 2, 10 Anella Avenue, Castle Hill, NSW 2154, Australia) and expressed as $\mu mol\,mg^{-1}$ protein by comparing absorbance at 725 nm with that of a 4-coumarate standard curve.

4-Coumarate coenzyme A ligase (4-CL; EC 6.2.1.12.) activity was determined according to the method of Moerschbacher *et al.* (1988). A 50-$\mu$L aliquot of extract was mixed with 700 $\mu$L of 0·2 M phosphate buffer (pH 7·3) containing 20 mM $MgCl_2$, 2 mM adenosine triphosphate (ATP), 4 mM DTT, 0·4 mM 4-coumarate and 1·8 g glycerol (added fresh daily) and incubated at 30°C for 1 min. The reaction was started by the addition of 100 $\mu$L of 0·2 M phosphate buffer containing 2 mM CoA. The absorbance at 333 nm was read immediately and after a 10-min incubation at 30°C. The activity of 4-CL was calculated as the increase in absorbance over 10 min and expressed as $pkat\,mg^{-1}$ protein using the extinction coefficient of 4-coumaryl: CoA ($2{\cdot}3\times10^7\ cm^2\,mol^{-1}$).

Cinnamyl alcohol dehydrogenase (CAD; EC 1.1.1.149.) activity was determined according to Moerschbacher *et al.* (1988). A 100-$\mu$L aliquot of root extract was mixed with 400 $\mu$L of 0·2 M Tris-HCl (pH 9·25) containing 0·3 mM $NADP^+$ (added fresh daily) and incubated at 30°C for 1 min. The reaction was started by the addition of 100 $\mu$L of 0·2 M Tris-HCl

containing 2 mM coniferyl alcohol (added fresh daily). The absorbance at 400 nm was read immediately and after a 10-min incubation at 30°C. CAD activity was calculated as the increase in absorbance over 10 min and expressed in pkat $mg^{-1}$ protein using the coniferyl aldehyde extinction coefficient of $2{\cdot}0 \times 10^7$ $cm^2$ $mol^{-1}$.

### Phosphite analysis

White root material was collected for phosphite analysis at each harvest and dried at 60°C for 5 days (approximate sample size 50 mg dry weight (DW). The dried root material was powdered in a mortar and pestle and placed in 10-mL centrifuge tubes containing 5 mL water for 24 h. The material was filtered and analysed by HPIC using methodology outlined by Roos *et al.*, 1999) using a Waters Millipore system equipped with a Vydac2 column. The amount of phosphite detected was expressed as mg $g^{-1}$ DW.

## Results

### Effect of phosphite on general plant health

At the time of phosphite application, the plants showed no symptoms of nutrient imbalance. After treatment with 5 g $L^{-1}$ phosphite, the leaves showed slight marginal and tip discoloration (within 1–2 days of treatment), which developed into burning (within 5–7 days of treatment). Young leaves were burnt by the phosphite at this concentration; however, by day 14 new growth was evident. Phosphite concentration increased in the roots with increasing time after application (Fig. 1a).

### Root growth and lesion development

The application of phosphite reduced ($P \leq 0{\cdot}05$) the rate of root extension by day 5 (Fig. 1b). When phosphite concentrations in the roots were highest (day 14; 3·2 mg $g^{-1}$ DW) the rate of root extension was lower than in the nonsprayed (day −4) plants (Fig. 1a,b). Inoculation also reduced root extension, to a rate below that caused by phosphite application (Fig. 2).

Phosphite application reduced lesion development in roots inoculated with *P. cinnamomi*. Lesion development decreased (Fig. 1c) as the phosphite concentration within the roots of the tolerant jarrah plants increased over the duration of the experiment (Fig. 1a). However, there was no statistical correlation between phosphite concentration and lesion development ($r^2 = 0{\cdot}2$). The reduction in lesion development was most effective in the roots inoculated between 8 and 14 days after the phosphite application (Fig. 1c). Where no visible lesions were evident, plating these roots onto a *Phytophthora*-selective medium confirmed the absence of the pathogen.

### Host defence enzymes

The levels of soluble phenolics and the activity of CAD in the roots of nontreated, noninoculated jarrah generally increased with distance from the root tip (Fig. 3b,c). In comparison, the levels of 4-CL activity generally decreased with increasing distance from the root tip (Fig. 3a).

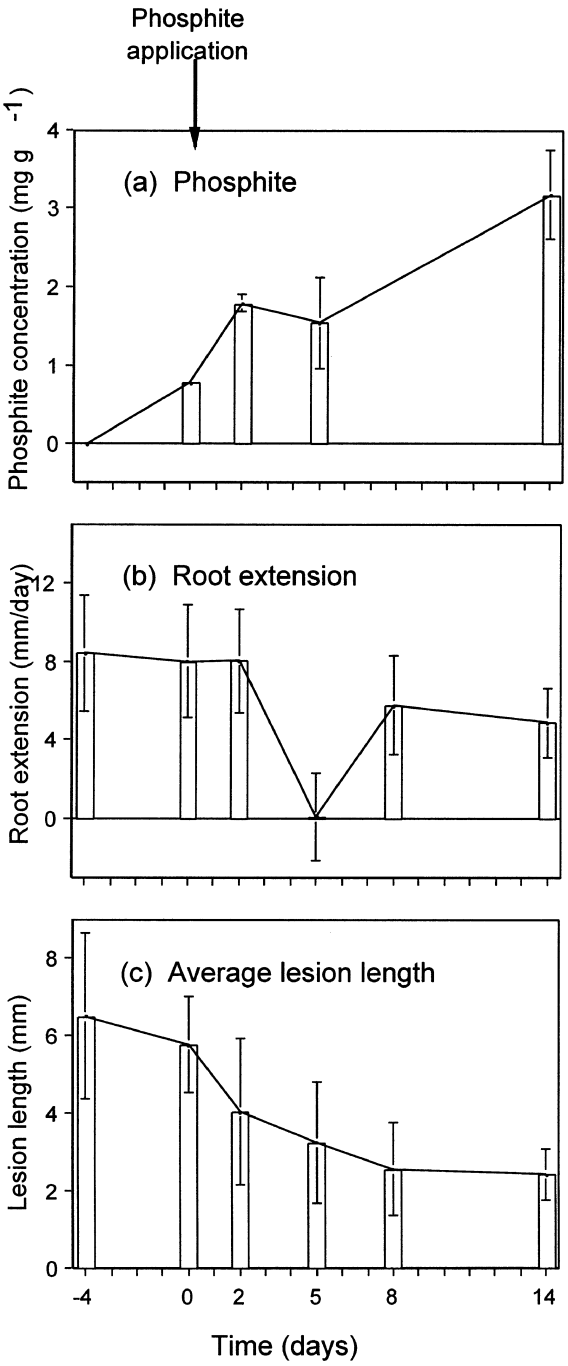


Figure 1 (a) Phosphite concentration (mg $g^{-1}$ DW), (b) rate of root extension (mm $day^{-1}$ in noninoculated roots), and (c) lesion length (mm) in the roots of *Eucalyptus marginata* inoculated 4 days prior to and 0, 2, 5, 8 and 14 days after a foliar application of 5 g $L^{-1}$ phosphite. Roots were harvested for assessment of root extension, lesion development and phosphite concentration 4 days after each inoculation event. Bars indicate the standard error of the mean.

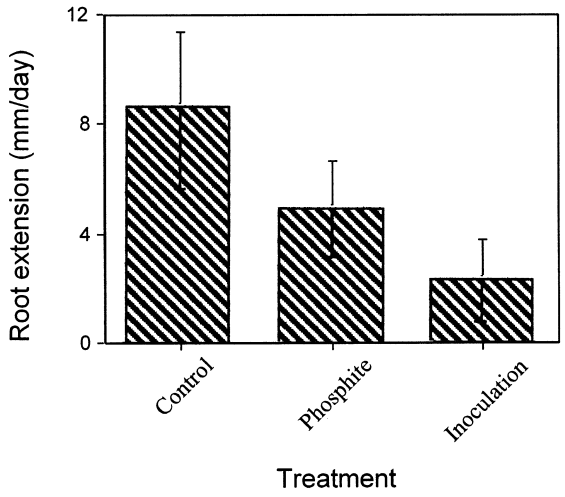


Figure 2 Average rate of root extension (mm day$^{-1}$) (bars indicate SD) of *Eucalyptus marginata* in control plants and after treatment with 5 g L$^{-1}$ phosphite or inoculation with *Phytophthora cinnamomi*.

### Effect of phosphite only

In noninoculated plants, phosphite accumulated in the white root (Fig. 1a). However, there was no change ($P \leq 0{\cdot}05$) in enzyme activity or phenolic accumulation in the root tip (Fig. 4).

### Effect of inoculation only

The levels of 4-CL activity increased after inoculation and remained relatively constant throughout the length of the inoculated roots (Fig. 3a). Recorded CAD activity was higher behind the lesion (segments 2 and 3) after inoculation, but this increase was not significant (Fig. 3b). In comparison, the total levels of phenolics increased ($P \leq 0{\cdot}05$) in root segments 2 and 3 after inoculation with *P. cinnamomi* (Fig. 3c).

### Interaction between inoculation and phosphite

The activity of 4-CL increased ($P \leq 0{\cdot}05$) in the roots of plants inoculated 5 days after phosphite application (Fig. 5a). CAD activity and accumulation of phenolics increased ($P \leq 0{\cdot}05$) in the roots of plants inoculated 2 and 5 days after phosphite application (Fig. 5b,c). At these times, the phosphite concentration within the roots was low (1·8 and 1·5 mg g$^{-1}$ DW, respectively) (Fig. 1a). When the phosphite concentration in the roots was highest (day 14; 3·2 mg g DW$^{-1}$) (Fig. 1a) the activity of 4-CL and CAD and the accumulation of soluble phenolics was low (Fig. 5b,c).

## Discussion

The foliar application of phosphite effectively restricted *P. cinnamomi* lesion development in the roots of the jarrah clone with moderate resistance to *P. cinnamomi*.

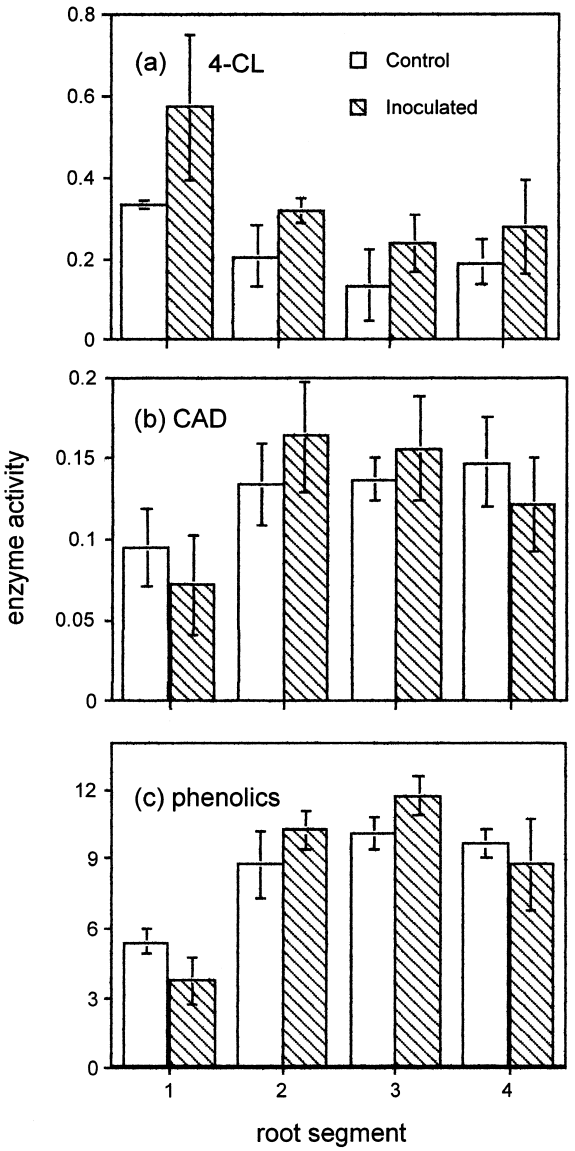


Figure 3 Activity of (a) 4-coumarate CoA ligase (4-CL), (b) cinnamyl alcohol dehydrogenase (CAD) (pkat mg$^{-1}$ protein), and (c) soluble phenolic accumulation (mmol mg$^{-1}$ protein) in the roots of *Eucalyptus marginata* plants, prior to and after inoculation with *Phytophthora cinnamomi*. The first root segment contains the root tip and the lesion (if present), subsequent segments are 2 cm in length. Control, before inoculation; inoculated, after inoculation. Bars indicate the standard error of the mean.

Lesion development was most effectively restricted in roots inoculated 14 days after phosphite application.

The application of phosphite reduced the rate of root extension for the first 5 days, after which root extension rate increased and stabilized below the levels recorded in nonphosphite-treated plants. This initial reduction in root extension appears to be a result of re-enforcement of the root tip and may reduce susceptibility of the roots to pathogen invasion. Guest (1986) reported a decrease in root growth and associated carbohydrate leakage, and

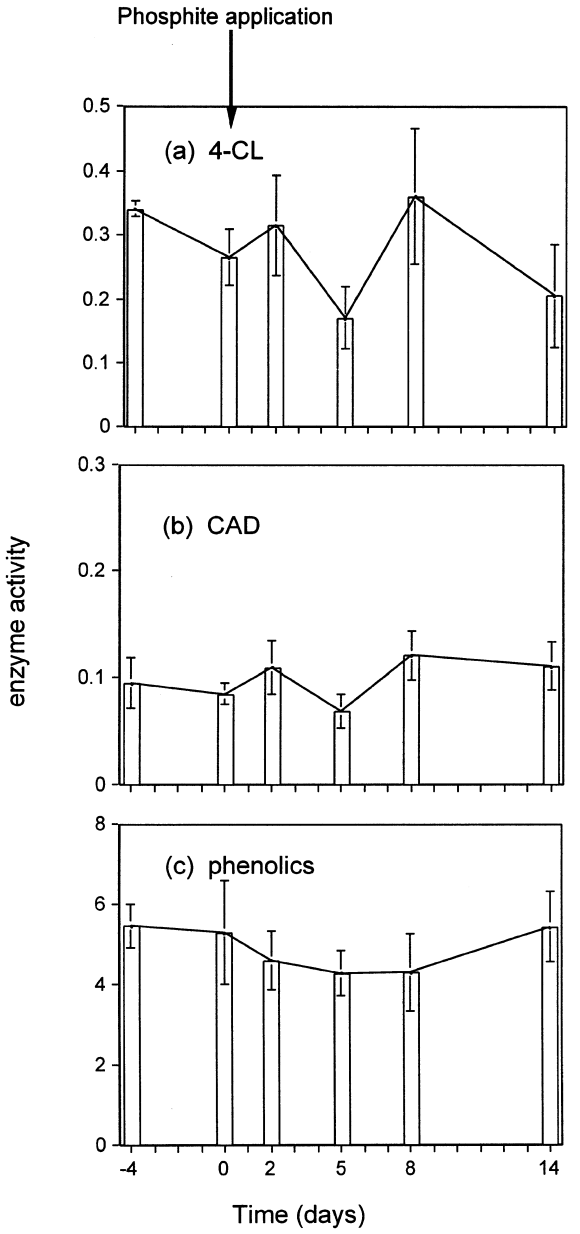


Figure 4 Activity of (a) 4-coumarate CoA ligase (4-CL), (b) cinnamyl alcohol dehydrogenase (CAD) (pkat $mg^{-1}$ protein), and (c) soluble phenolic accumulation (mmol $mg^{-1}$ protein) in the root tips of noninoculated *Eucalyptus marginata*, 4 days prior to and 0, 2, 5, 8 and 14 days after foliar application of 5 g $L^{-1}$ phosphite. Bars indicate the standard error of the mean.

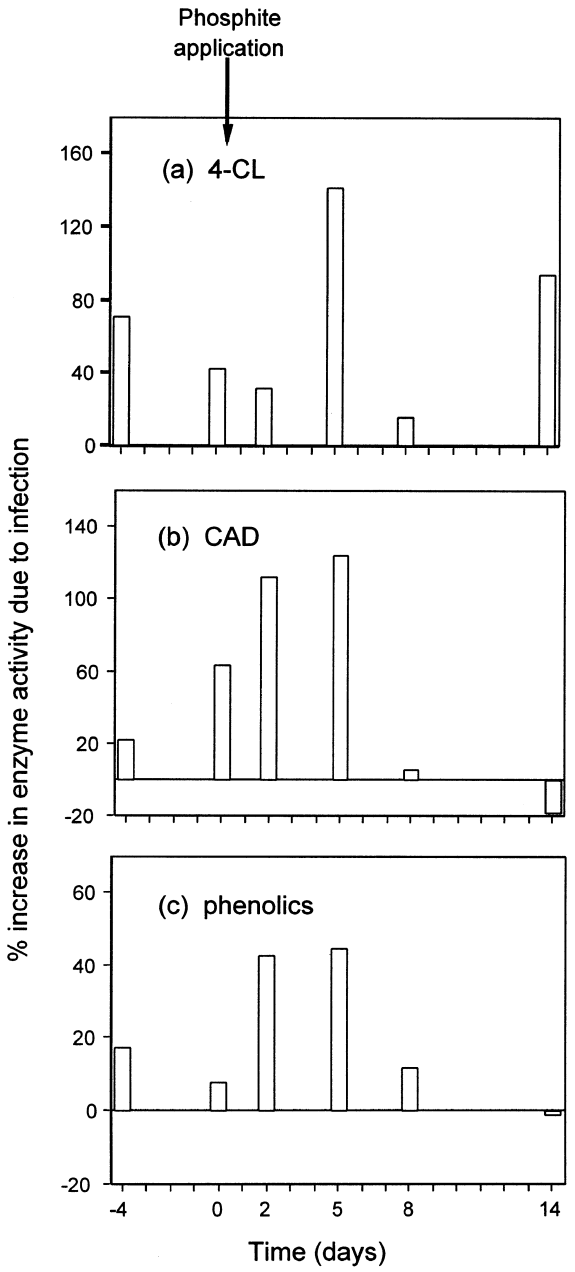


Figure 5 Percentage change in (a) 4-coumarate CoA ligase (4-CL), (b) cinnamyl alcohol dehydrogenase (CAD) activity (pkat $mg^{-1}$ protein), and (c) phenolic accumulation (mmol $mg^{-1}$ protein) in the second root segment of *Phytophthora cinnamomi* tolerant *Eucalyptus marginata* inoculated with *P. cinnamomi* 4 days before and 0, 2, 5, 8 and 14 days after foliar application of 5 g $L^{-1}$ phosphite. Bars indicate the standard error of the mean.

reduced root transpiration as a result of treatment with Fosetyl-Al®. Studies by Jackson (1997) showed that phytotoxic phosphite concentrations (15 g $L^{-1}$) induced a darkening of the root tip, which was thought to be associated with soluble phenolic accumulation.

Phosphite is translocated throughout the plant in a source–sink relationship in association with photoassimilates (Ouimette & Coffey, 1990; Guest & Grant, 1991). The interval between phosphite treatment and inoculation influences the levels of enzyme activity and phenolic accumulation associated with lesion development. Therefore, the effectiveness of phosphite in protecting the roots from infection by *P. cinnamomi* depends on the rate at which it is translocated to the roots after foliar application. In the current study, phosphite concentrations in the roots were highest

14 days after foliar application of 5 g $L^{-1}$ phosphite. At this time, lesion development was significantly reduced compared with nonphosphite-treated plants. However, although lesion development was effectively restricted, the levels of host defence mechanisms remained relatively unchanged compared with those of nonphosphite-treated, noninoculated plants. In comparison, the activity of host defence enzymes (4-CL and CAD) and the accumulation of soluble phenolics peaked in roots of plants inoculated 2 and 5 days after phosphite application. During this period, lesion development was reduced (compared with nonphosphite-treated roots), although not as effectively as in roots inoculated 14 days after phosphite treatment. Phosphite concentrations within the roots were low (compared with levels at day 14) 2 and 5 days after phosphite application. Therefore, lesion development was restricted predominantly via the indirect action of phosphite on the pathogen in inducing host defences. In contrast, 8 and 14 days after phosphite application, phosphite concentrations were high within the roots. At this time, lesion development was reduced predominantly via the direct action of the phosphite in inhibiting mycelial growth, thereby suppressing the pathogen before it could establish an association with the host.

Previous studies have monitored timing of enzyme induction after phosphite treatment and inoculation; however, enzyme activity was only measured at a single inoculation interval after phosphite treatment (Guest, 1984; Saindrenan *et al*., 1988; Nemestothy & Guest, 1990). Recent studies on moderately resistant jarrah have shown that the foliar application of a phytotoxic phosphite concentration (15 g $L^{-1}$) provided maximum protection of the roots from pathogen invasion 7–10 days after its application, at which time no lesions were observed. During this time there was little change in the levels of host defence enzymes. The 15 g $L^{-1}$ phosphite treatment also reduced lesion development in roots inoculated 2 and 77 days after phosphite treatment, compared with nonphosphite-treated plants. An induction of host defence responses was associated with the reduction in lesion development (Jackson, 1997). Phosphite concentrations within the roots 7–10 days after application were thought to be high and therefore the mode of phosphite action in restricting mycelial growth was considered to be predominantly via direct inhibition of the pathogen. The efficient induction of host defence mechanisms in roots inoculated 2 and 77 days after phosphite application was attributed to the indirect action of phosphite at low concentrations interacting with the pathogen to stimulate host defences. The results of the current study suggest that phosphite concentration at the site of pathogen invasion affects the mode of phosphite action in controlling the pathogen within the host.

The method used for determining phosphite concentrations in the roots at various time periods after foliar application is not an accurate measure of the concentration of phosphite faced by the pathogen at the site of infection. The average phosphite concentration determined by tissue analysis may be significantly different from the actual phosphite concentration in intercellular fluid, vacuoles and so on. Therefore, further studies are required to determine where the phosphite is translocated within the tissues, where it is stored and in what concentrations it is stored. This information will lead to a better understanding of the concentrations of phosphite required to inhibit pathogen growth *in vivo*.

When phosphite concentrations within the roots are low, the action of phosphite in reducing lesion development is predominantly via an interaction with the pathogen that stimulates the hosts defences. Perez *et al*. (1995) demonstrated that low levels of phosphite disrupt the metabolism of the pathogen, altering the cell wall structure, which results in the release of elicitors leading to an induction of plant defence mechanisms. The degree to which the metabolism is disrupted depends on the tolerance of the pathogen to phosphite as well as the resistance of the host to the invading pathogen. At high phosphite concentrations, however, phosphite acts directly on the pathogen, inhibiting its growth before it can establish an association with the host, and therefore the host defence mechanisms remain unchanged.

In conclusion, the mode of phosphite action, whether direct or indirect, in controlling *P. cinnamomi* in clonal jarrah, depends on a combination of (a) the time interval between phosphite treatment and inoculation; (b) the concentration of phosphite applied; and (c) the tolerance of the host and the pathogen to phosphite. Further studies are required in order to determine the length of time for which phosphite protects the plant against pathogen invasion. In addition, further research is required to establish a more complete understanding of the inter-relationship between the host and pathogen in the presence of phosphite.

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