Effects of phosphite on disease development and histological responses in *Eucalyptus marginata* infected with *Phytophthora cinnamomi*

by

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Declaration

I declare that this thesis is my own account of my research and contains as its main content, work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

Phosphite is currently used for the management of *Phytophthora cinnamomi* in native plant communities. A greater understanding of how phosphite affects the host-pathogen interaction is required in order to determine the most effective treatment. This thesis aimed to investigate the effects of applied phosphite concentration on phytotoxicity, *in planta* concentration of phosphite, disease development and anatomical responses of *Eucalyptus marginata*.

Spraying the foliage to run-off with 7.5 and 10 g phosphite/L led to the development of severe leaf necrosis within 7 days, with greater than 60% of the leaf area damaged. Moderate phytotoxicity was observed after treatment with 5 g phosphite/L. *In planta* concentration of phosphite in stems, lignotubers and roots did not differ significantly between applied concentrations of phosphite. Stem tissue contained the largest concentration of phosphite at one week after spraying, with approximately 210 and 420 µg phosphite/g dry weight detected after treatment with 5 and 10 g phosphite/L, respectively.

In a subsequent field trial, the applied concentration of phosphite was found to affect the duration of effectiveness of phosphite in protecting *E. marginata* seedlings from stem colonisation by *P. cinnamomi*. Plants were wound-inoculated with *P. cinnamomi* at 6-monthly intervals after spraying with phosphite. The 2.5 and 5 g phosphite/L treatments were effective against colonisation by *P. cinnamomi* when inoculated 0 and 6 months after spraying, but only the 5 g phosphite/L treatment inhibited *P. cinnamomi* within 12 months of spraying. Phosphite had no effect on colonisation by *P. cinnamomi* when plants were inoculated at 17 months after spraying. The *in planta* concentration of phosphite detected in the leaves, stems and roots of plants treated with 5 g phosphite/L did not differ significantly between the time of harvest or tissue type at 0.2 and 6 months after spraying. *P. cinnamomi* remained viable in plants treated with phosphite.

Treatment with 2.5 and 5 g phosphite/L when *P. cinnamomi* was well-established in the stems was ineffective at preventing the death of *E. marginata*. Between 45 and 89% of plants were girdled on the day of spraying. Spraying plants with 2.5 and 5 g phosphite/L when conditions were less favourable for the pathogen reduced the mortality of *E. marginata* for up to 10 months.

E. marginata seedlings responded to damage by *P. cinnamomi* with the production of kino veins and woundwood. Bark lesions were in the process of being sloughed off by 7 months after inoculation in plants that remained alive.

In plants of a resistant (RR) clonal line and susceptible (SS) clonal line, phosphite treatment inhibited lesion extension in stems, but lesions did not indicate the amount of stem colonised by *P. cinnamomi*. The pathogen was isolated from up to 17 cm beyond the lesion front in the RR clonal line. Treatments that reduced the mortality of *E. marginata* were 5 g phosphite/L in the RR clonal line (RR/5) and 10 g phosphite/L in the SS clonal line (SS/10).

Uninoculated plants were wounded with liquid nitrogen to determine the microscopic responses to injury in the absence of the pathogen. Wound closure was achieved within 21 days of wounding, with callus formation and vascular cambium regeneration. A wound periderm separated wounded tissue from healthy tissue, adjacent to a lignified boundary zone. Two types of phellem were observed – thin-walled phellem (TnP) and thick-walled phellem (TkP). The first-formed TnP layers contained variable-shaped cells, while subsequent layers were more cubical in shape. Multiple TnP layers developed up to 42 days after wounding, with TkP cells sandwiched between the TnP layers. Genotype and phosphite treatment did not affect the wound responses.

Inoculated plants with a restricted lesion extension also formed a wound periderm to separate damaged tissue from healthy tissue. Phosphite treatment stimulated the responses to *P. cinnamomi* in both clonal lines. Early development of the wound periderm was visible by 6 days after phosphite treatment. It was

preceded by the formation of a ligno-suberised boundary zone in the cambial zone and in phloem parenchyma cells existing prior to injury. Suberin was not detected in the SS/0 treatment. TnP layers completely surrounded lesioned tissue in plants still alive by 24 days after phosphite treatment. Extensive callus production was evident in the SS/10, RR/5 and RR/10 treatments.

Temperature affected the post-inoculation efficacy of phosphite and anatomical responses of *E. marginata*. At 20°C, lesion extension was restricted in both clonal lines of *E. marginata*, irrespective of phosphite treatment. Greater than 70% of inoculated plants in all treatments produced a ligno-suberised boundary zone at 20°C and between 30 and 70% formed a wound periderm. At 28°C, lesion extension was reduced in phosphite-treated plants at 7 days after treatment. However, lesions continued to extend up to 5 mm per day in the SS clonal line and very few SS plants formed a wound periderm at the lesion front. This contrasted with the strong responses to abiotic wounding observed in uninoculated SS plants at 28°C. The most extensive responses to *P. cinnamomi* were detected in the RR/5 treatment at 28°C, with a ligno-suberised boundary zone and differentiated TnP of a wound periderm observed in greater than 70% of plants. This treatment resulted in significantly less girdled plants than all other treatments at 28°C, including the RR/0 treatment. At 23 and 24°C, there was no significant difference in acropetal lesion extension or circumferential lesion spread between clonal lines. The inoculation technique and environmental conditions may have resulted in too high a disease pressure for a full expression of resistance in the RR clonal line.

This thesis demonstrates that phosphite has the potential to enhance the resistance of young *E. marginata* and enable them to survive infection by *P. cinnamomi*. However, its effectiveness is dependent upon a number of factors, including host resistance, environmental conditions, the applied phosphite concentration and the timing of application.

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