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ORIGINAL RESEARCH ARTICLE

Foliar phosphite application to manage kauri (*Agathis australis*) dieback

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Abstract

Phosphite (HPO_3^{2-}) is an effective chemical treatment used to manage *Phytophthora* diseases in natural and horticultural environments. In New Zealand it is applied as a trunk injection to protect the threatened endemic foundation tree, *Agathis australis* (kauri), from the invasive pathogen *P. agathidicida*. It is applied as a foliar spray in natural ecosystems in Australia to protect against a range of *Phytophthora*-associated diseases and retain plant biodiversity and abundance. Foliar spray application could be a useful method for broad area treatment and to protect isolated and difficult-to-reach stands of kauri, or in plant production settings. In this study, we assessed the efficacy of phosphite when applied as a foliar spray to inhibit *P. agathidicida* colonisation, the phytotoxicity response and whether the phosphite was translocated to the roots using two glasshouse experiments with 2- and 6-year-old kauri plants. The foliar phosphite treatment effectively reduced colonisation by *P. agathidicida* in the 2-year-old plants and elevated concentrations of phosphite in the roots of the 6-year-old plants were detected. Phytotoxicity increased with increasing phosphite concentration in both trials. The addition of the surfactant Du-Wett® reduced phytotoxicity for older leaves in the 6-year-old plants. In both assays, phytotoxicity symptoms were most severe on young leaves but had no effect on the production of fresh growth following treatment, suggesting that any ill effects were short-lived. This study indicates that phosphite foliar sprays may be an effective tool to manage kauri dieback under the appropriate circumstances.

Keywords *Phytophthora agathidicida* · Phosphite · Foliar spray · *Agathis australis*

Introduction

The genus *Phytophthora* is associated with several diseases of keystone forest tree species, including oak (*Quercus* spp.) (Jung et al. 2000) and beech (*Fagus sylvatica*) (Jung 2009) in Europe, and jarrah (*Eucalyptus marginata*) in Australia (Podger 1972). In New Zealand, kauri (*Agathis australis*) suffers from a dieback disease primarily caused by the invasive pathogen *P. agathidicida* (Weir et al. 2015). Phosphite (HPO_3^{2-}), the biodegradable systemic fungicide (Guest and Grant 1991), is currently applied to kauri as trunk injections to manage kauri dieback (Horner et al. 2015). Phosphite accumulates in the meristematic tissues in concentrations high enough to inhibit the growth of oomycetes (Smillie et al. 1989; Ouimette and Coffey 1990) while stimulating host defence responses (Smillie et al. 1989; Eshraghi et al. 2011; Kasuga et al. 2021). It is of interest to disease management to determine if foliar sprays could be used to complement trunk injections to manage *P. agathidicida* in kauri. The aim of this study was to determine if phosphite is translocated to the roots of kauri following foliar application and confirm the role of phosphite in inhibiting colonisation of *P. agathidicida* through trunk inoculation.

Phosphite has been shown to be a suitable treatment for use in natural environments (Hardy et al. 2001). The

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complex mode of action of phosphite triggers several biochemical mechanisms meaning that while it is persistent in plants, it is ephemeral in the environment, leaving no toxic residues (Guest and Grant 1991). In addition, a single application of phosphite has been demonstrated to have an effect for several years (Shearer and Fairman 2007; Horner et al. 2015; Horner 2020).

Phosphite is highly inhibitory to *P. agathidicida* mycelium (Horner and Hough 2013; Hunter et al. 2022) and reduces the expansion of oozing basal trunk lesions, in treated forest trees (Horner et al. 2015). Phosphite is currently injected into kauri trees at recommended rates of 4 or 6% at either 25 or 40 cm spacing around the trunk (Horner 2020). Single injections of phosphite have been shown to be effective for at least 10 years (Horner et al. 2017; Horner 2020; Horner et al. 2024), but the scale of application is limited by the accessibility of sites, the amount people can carry into the forest and the number of applications that can be completed in one session (Hardy et al. 2001). Holes must be drilled for every injection point and the injection is passively taken up into the tree, so it requires time. Training and upskilling are required of contractors for treating the trees and for working in infected forests to avoid spreading the pathogen. Due to its systemic nature, foliar-applied phosphite is suitable for protecting plants from soil-borne *Phytophthora* pathogens, though treatments must be optimised for different hosts and environments and different ages of host trees.

Phosphite application has been used to protect native Australian flora from *P. cinnamomi* since 1997; regular foliar applications of between 12 and 24 kg ha^{-1} have been applied to native woodland and shrubland in the southwest Floristic Region of Western Australia (Hardy et al. 2001; Barrett and Rathbone 2018). Due to the vast host range of *P. cinnamomi* in Western Australia of more than 2000 plant species (Shearer et al. 2004), foliar application is a time and cost-effective method to protect whole plant communities. Plants vary in their responses to phosphite aerial sprays with up to 28-fold differences in *in planta* phosphite concentrations and effectiveness between taxa (Shearer et al. 2012).

On a community level, the phosphite regime in Western Australia has maintained species assemblages, richness and structure that would otherwise be declining due to *P. cinnamomi* (Barrett and Rathbone 2018). When applying a foliar spray, multiple hosts can be covered within the ecosystem including other asymptomatic host species that could be carriers for the pathogen. For example, Shearer and Crane (2012) have shown that aerial phosphite application reduced the infection rate in plant species that do not effectively take up phosphite, by reducing the inoculum load in the surrounding plants. At the forest stand level, foliar sprays are the most scalable and effective application method to treat emergent and canopy tree species (Hardy et al. 2001; Solla et al. 2021). Aerial application of phosphite has the potential to save time and money in treating large trees in forests while minimising forest disturbance, risks of pathogen spread and physical damage necessary for trunk injections.

Recently, trials were run in natural forest in Poland (*Q. robur*) and Spain (*Q. ilex*) to test the effectiveness of phosphite in controlling oak decline caused by soilborne *Phytophthora* species (Solla et al. 2021). Phosphite was applied as trunk and foliar sprays and ultra-low volume aerial sprays (3 – 4 L ha^{-1}). The aerial and trunk sprays applied at 35% (237.5 g of phosphorous acid per litre) resulted in significant improvements in crown conditions in relation to controls. Foliar sprays (0.28 to 1.12% phosphite) had no effect on the crown condition of trees; however, no surfactant was added. Low concentrations were used because *Q. ilex* in this area of Spain is highly phosphate (P) deficient, and phosphite can cause phytotoxicity in P-deprived trees.

To test whether foliar application of phosphite would be useful to manage kauri dieback, it is necessary to compare formulations of phosphite and adjuvants, the uptake and phytotoxicity response of different leaf ages, whether it is translocated to the roots and if it inhibits *P. agathidicida* colonisation. Three studies have tested foliar application of phosphite on potted kauri saplings; they tested efficacy to inhibit *P. agathidicida* colonisation (Horner and Hough 2013), phytotoxicity (Scott et al. 2016) and uptake (Horgan 2017). In the first study, foliar application was compared to trunk injections and soil drenches on 2-year-old potted kauri infected with *P. agathidicida* (Horner and Hough 2013). Injections were the most effective in restoring plant health, however, no adjuvants were added to aid the solution adherence to the leaf surface and uptake, which is critical when applying phosphite aerially (Hardy et al. 2001). This is likely to have contributed to the low success of foliar application in the reported glasshouse trial (Horner and Hough 2013).

The second study conducted a phytotoxicity trial with 1.5-year-old kauri saplings and plants commonly found in kauri forests, including *Asplenium bulbiferum, Beilschmiedia tawa, Cordyline australis, Dacrydium cupressinum, Kunzea ericoides, Leptospermum scoparium, Podocarpus totara* and *Prumnopitys ferruginea* (Scott et al. 2016). All plants experienced very little phytotoxicity to 12, 24 and 48 kg a.i. ha^{-1} of phosphite containing 0.2% Du-Wett® (Elliott Chemicals Ltd, Auckland, New Zealand; (Scott et al. 2016). It was concluded that foliar phosphite applications were suitable in the natural environment in New Zealand (Scott et al. 2016). Optimising application rates and timing for a targeted application regime should further minimise phytotoxicity (Scott et al. 2016).

The final trial tested the uptake of three commercial formulations of phosphite (Agrifos® 600 (Key Industries, Auckland, New Zealand), Foschek® (Arxada Ltd, New Zealand), and Phosgard™ (Growchem, New Zealand) at

12 kg/ha with adjuvants (Du-Wett®, Actiwett® and Break-Thru® SP131) on the abaxial and adaxial surfaces of kauri saplings applied by microsyringe (Horgan 2017). Phosphite uptake was higher from the abaxial (lower) surfaces (> 65%) compared to the adaxial (upper) (< 5%) (Horgan 2017). The adaxial side of kauri leaves has 2 rows of cells in the hypodermis, mostly of thick-walled tracheids, while the abaxial side has a single row of dimorphous tracheids which are discontinuous (Vasilieva 1972). The presence of stomata and thinness of the hypodermis of the abaxial side probably contributed to greater uptake. Foschek® and Phosgard™ had good uptake without adjuvants and the addition of adjuvants did not make any significant improvements in uptake (Horgan 2017). Agrifos® 600 had poor uptake (~ 5%), this formulation is currently used for trunk injections of kauri (Horner et al. 2015) and the only one tested in the initial inhibition assays to compare application methods (Horner and Hough 2013).

It is unlikely that phosphite itself will cause phytotoxicity to kauri based on the results from Scott et al. (2016), but it is important to test the phytotoxicity of adjuvants themselves (Hardy et al. 2001). While adjuvants did not increase the uptake of phosphite formulations in the study by Horgan (2017), their addition promotes spray retention and increases coverage by spreading droplets while also decreasing spray drift, evaporation and wash-off (Hardy et al. 2001). To understand if phosphite foliar sprays would be effective in protecting kauri from *P. agathidicida*, it is necessary to test uptake, phytotoxicity (to formulations and adjuvants), and translocation in a combined comparative study.

The aims of this study were to:

1) determine if foliar applied phosphite is translocated to the roots of 6-year-old kauri plants,
2) demonstrate whether foliar applied phosphite inhibits colonisation of *P. agathidicida* of trunk inoculated 2-year-old kauri plants, and;
3) determine if foliar applied phosphite results in phytotoxicity in potted kauri plants.

Methods

Inhibition of *Phytophthora agathidicida* colonisation and phytotoxicity impacts in 2-year-old kauri treated with foliar applied phosphite

Agathis australis growth and maintenance

Agathis australis (kauri) seedlings were sourced from Scion Research Limited from seeds collected from the Waitākere Ranges Regional Park with permission from Te Kawerau ā Maki, the local iwi (tribe) that manage the forest. These were initially germinated into seedling trays where they were held by competitive inhibition to maintain a small plant and were repotted 2 years prior to the experiment. The plants were gifted to Plant and Food Research Limited, Havelock North New Zealand in 2018 and maintained in a glasshouse. A soil containing minimal additional fertiliser, apple cutting mix, (GroCom, Hastings, New Zealand) was pasteurised at 70 °C for 60 min. Kauri seedlings were potted into clean PB2 bags or autoclaved 9 × 9 × 15 cm pots.

Seedlings were watered soon after potting into soil and placed into a glasshouse with shade cloth and manual venting with temperatures ranging from 30 °C in summer to 5 °C in the winter. Nitrosol liquid fertiliser (Nitrosol, Auckland, New Zealand) was applied to the plants on 30/08/22 at the recommended label rate. Watering was done twice weekly until inoculation and throughout the experiment from October – December 2022.

The plants were moved to the glasshouse prior to inoculation, and the air conditioning was set to 20 °C (ranging from 8.5 to 33.5 °C). At the start of the experiment, the plants were 2 years old, ranged from 18 to 40 cm high (from the soil level) and had a trunk diameter of 34 to 65 mm.

Experimental design

This experiment consisted of two trials, an inoculation trial and a phytotoxicity monitoring trial which were run in parallel under the same glasshouse conditions (details below). Both trials were established with 2-year-old kauri plants arranged in a randomized complete block design. Each treatment had 6 replicates. In the inoculation trial 0, 5, and 7.5 g/L phosphite concentrations were used, in the phytotoxicity trial an additional treatment of 10 g/L was used. The replicate block numbers were randomly assigned using a random number generator. The pots were in a plastic bag to prevent water loss from soil evaporation for a parallel physiology study (data not presented).

As the size of plants and pots available was variable, plants were first sorted from smallest to largest trunk diameter in the two-pot type groups, with those of similar size grouped by replicate blocks, which were spread across the treatments. The two pot types were clean PB2 (1.15 L) planter bags or autoclaved 9 × 9 × 15 cm (1.1 L) pots. Treatment and position within the block were then allocated in a randomized split-plot design.

Inoculation trial

Inoculum production Whole oat grains were soaked overnight in 500 mL of deionised water and then autoclaved twice, leaving a day between each autoclaving. Oats were inoculated separately with four *P. agathidicida* isolates from the Hawkes Bay, Plant and Food Research, *Phytophthora*

collection; H1478, H1436, H1325 and H1650. The isolates were sourced from soils found in Mahurangi, Auckland region, Waitākere Ranges and Trounson Kauri Park, respectively. Six agar cubes from the leading edge of a 5-day-old culture on 20% V8 agar (80 mL V8 Vegetable juice, 320 mL deionised water, 6 g agar, 1 g $CaCO_3$) were used to inoculate the oats. Oats were inverted every three to four days to ensure even colonisation.

Phosphite treatment Preventative and curative treatments using three concentrations (0, 5 and 7.5 g/L) of Foschek® (400 g/L phosphorous acid present as mono-di potassium phosphite) and 0.2% of the non-ionic organosilicone surfactant Du-Wett® were applied four days prior and two days following the inoculation of the plants, respectively. Treatments were applied to the foliage of each treated plant to run off. To prevent the phosphite from leaching down the trunk and into the soil, a rolled paper towel was wrapped around the trunk at 5 cm above the soil level and a plastic bag was secured to it as a skirt. An additional treatment of 7.5 g/L without Du-Wett® was included in the preventative treatment group to assess the effect of the surfactant. A control treatment in which the plants were sprayed with water and inoculated with a sterile oat grain was included. There were a total of 42 plants in this trial across the seven treatments (0 g/L inoculated, 0 g/L uninoculated control, 5 g/L preventative and curative and 7.5 g/L preventative and curative all with Duwett® and 7.5 g/L preventative treatment without Duwett®).

Inoculation Trunks were inoculated by cutting on a gentle angle into the cambium 40 mm above the soil surface and placing a colonised oat grain under the flap (based on Horner and Hough 2014). This was held in place and sealed with Parafilm® (Bemis Company, Wisconsin, USA). Control trees were inoculated with a sterile oat. Inoculations and treatments were established in a randomized complete block design.

Harvest and measurement A foliage health scale modelled off Horner and Hough (2013) was used to track symptom expression. The scoring system was as follows 0 = healthy, 1 = slight wilt, 2 = moderate wilt, 3 = severe wilt. Once 50% of the trees began to wilt severely (health score 2.5 or 3) plants were all harvested. Wilt scores were taken 9, 15 and 18 Days Post Inoculation (DPI) and the plants were harvested at 18 DPI.

To harvest the plants, they were cut at the soil level, and side branches and leaves were removed to make the inoculation area accessible. The trunk was surface sterilised using a paper towel dampened with 80% ethanol and allowed to dry. It was cut longitudinally through the centre of the inoculation point and in half perpendicularly through the centre of the inoculation point. Four 5 mm fragments below and above the centre of the lesion were plated onto PARPH (a *Phytophthora*-selective media containing 10 mg/L Pimaricin, 100 mg/L Ampicillin, 10 mg/L Rifamicin and 75 mg/L PCNB in cornmeal agar) in order. The trunk was cut in half longitudinally to make plating the fragments easier. The plates were checked for growth 3 and 6 days after plating. Growths were identified based on hyphae morphology and oospore formation.

Phytotoxicity monitoring trial

In addition to the inoculated plant trial, another trial was run in parallel in which plants were treated to assess phytotoxicity in response to phosphite at four concentrations (0, 5, 7.5 and 10 g/L) of Foschek® with 0.2% Du-Wett®. An additional treatment of 7.5 g/L without Du-Wett® was included. These plants were not inoculated with *P. agathidicida*. There was a total of 30 plants in this trial, 6 replicates for each of the 5 treatments. Treatments were applied to the foliage of each treated plant to run off. To prevent the phosphite from leaching down the trunk and into the soil, a rolled paper towel was wrapped around the trunk at 5 cm above the soil level and a plastic bag was secured to it as a skirt.

To sort the plants, they were grouped into three size classes (small, medium, and large) for each of the two pot types (PB2 bag or 9 × 9 × 15 cm pot) and one plant from each size class for each pot type combined to make a treatment group (n = 6). All plants ranged from 10 to 26 cm in height (to the tallest part of growth, not necessarily the meristem). Plants with new growth were spread across the treatment blocks so that all treatments would be used on fresh growth and > 1 year-old growth. Treatments were randomized by size classes into each replicate block.

Phytotoxicity was noted as a binary measurement of presence or absence for leaves < 1 year old and ≥ 1 year old. The number of symptomatic leaves (showing water marks, burning and/or chlorosis) was also recorded. Phytotoxicity was monitored for every plant 7 days after the curative treatments were applied. Observations of both fresh growth and phytotoxicity were noted 16 days after the curative treatment.

Translocation of foliar applied phosphite in 6-year-old kauri and phytotoxicity response

Agathis australis growth and maintenance

The plants were grown from seed sourced from Colin Machon House, Titirangi, Auckland, New Zealand and were 6 years old at the start of the experiment. The plants were maintained in the glasshouse with a fan and heater set to 20 °C to maintain that temperature with shade cloth on

the roof. Plants were watered twice a week for the duration of the experiment.

Twenty-two plants were used in this trial, and they ranged from 105 – 154 cm in height and 40 – 52 mm in girth. Twelve of the plants had a flush of fresh growth at the start of the trial.

Experimental design

This experiment was established with 6-year-old kauri plants treated with phosphite at 0, 5, or 7.5 g/L phosphite, with or without the surfactant DuWett®. The treatments were randomly assigned using a random number generator while ensuring that all treatments would be applied to plants with fresh growth. The plants were arranged in a randomized complete block design. Each treatment had three or four replicates.

Phosphite treatment

Three concentrations (0, 5 and 7.5 g/L) of Foschek®, with and without Du-Wett® at a rate of 0.2% were applied between 6 and 7 am on 14/11/2023 (Table 1) as stomatal conductance (Macinnis-Ng et al. 2017) and sap flow (Bader and Leuzinger 2019) are highest in the early morning.

The phosphite treatments were mixed immediately prior to application. Plastic skirts were secured around the base of the trunks with a paper towel and masking tape to prevent the treatments from entering the soil. The plants were laid down horizontally with the foliage hanging off the glasshouse bench and sprayed to drip with 100 mL of treatment. The adaxial and abaxial surfaces of the leaves were thoroughly covered (Barrett 2001; Horgan 2017; Macinnis-Ng et al. 2017). Each plant was maintained in a separate tray to avoid cross-contamination of treatments and pseudo-replication. The plants were allowed to fully dry before returning them to their position in the glasshouse.

Table 1 Phosphite treatment groups for the large seedling glasshouse trial in Auckland

Treatment ID	Phosphite (g/L)	Du-Wett® (2%)	Replicates
A	0	yes	3
B	0	no	3
C	5	yes	4
D	5	no	4
E	7.5	yes	4
F	7.5	no	4

Phytotoxicity monitoring

Phytotoxicity was monitored 1-, 3-, 6- and 14-days post-treatment using the same binary method used in Sect. 6.2.3.7. The presence of fresh growth was noted at harvest for all plants 8 weeks after treatment.

Detached leaf assays

To determine if the phosphite treatments had been absorbed by the leaves, three detached leaf assays were conducted; the first at 24 h prior to phosphite treatment and at 2- and 4-weeks post-treatment. Three mature leaves from the previous flush of leaves were harvested from different branches at peak photosynthesis (2:30 pm) and carefully wiped with a damp tissue and stored in a plastic container with a moist paper towel and the lid sealed. Leaves that showed phytotoxicity symptoms (such as blisters or drying) were not selected. At each time point, one plant from each treatment (six plants total) was randomly selected and used as an uninoculated control (three leaves per plant). The boxes were transported to the lab for inoculation.

To inoculate, a sterilized needle was used to wound the leaf 1 cm from the proximal end on the abaxial leaf surface. A 7 mm diameter agar plug (grown on 10% clarified V8 agar) from the growing margin of a 7-day old colony of *P. agathidicida* (isolate ICMP18406 from the Landcare Research Culture collection, New Zealand) was placed upside down over the incision on each leaf. The uninoculated controls were inoculated with a sterile 7 mm V8 agar plug.

The detached leaves were stored in sealed containers at 20 °C in a 12 h light:12 h dark cycle under white LED lights with a dampened paper towel to prevent leaf drying. The paper towel was re-moistened as necessary. The leaves were harvested 10 days after inoculation and the adaxial surface photographed while laid next to a ruler. The percentage lesion area was quantified using ImageJ software program (version 1.54j; National Institutes of Health). The leaves were surface sterilised in 50% ethanol and rinsed in sterile deionised water twice and the lesion margins (two 5 mm segments) were plated onto PARPH to monitor for *P. agathidicida* growth.

Root phosphite concentration testing

Roots were harvested 8 weeks after phosphite treatment (on 9/1/2024). The three treatments at 0, 5 and 7.5 g/L with Du-Wett® (A, C and E from Table 1) were selected for root phosphite testing. Due to the cost of testing, roots were harvested from all replicates and combined into single samples for each treatment. A representative sample of fine feeder roots was collected from across the whole root zone, taking care to include

fresh growth nodules. Roots from each replicate were gently mixed together and wrapped in a dry paper towel then sealed in a plastic bag. At least 10 g was collected per treatment and sent to Hill Laboratories, Hamilton, New Zealand for phosphonate concentration testing. Phosphorous acid was extracted using aqueous extraction and solid phase extraction (SPE) cleanup and then quantified with liquid chromatography (LC) and mass spectrometry (MS) at mg/kg as received and mg/kg at 75% moisture content. The samples were submitted without indication of the phosphite treatment concentrations.

Data analysis

All data analyses were conducted using R version 4.3.3 (R Core Team 2023). Data were visualised with ggplot2 version 3.5.1 (Wickham 2016). The Shapiro–Wilk test was used to test for deviation from normality for all datasets (Shapiro and Francia 1972). The Levene test was used to compare variance between groups (r package car version 3.1–2). The data were not normally distributed, so non-parametric tests, including Kruskal–Wallis and Wilcoxon Pairwise, were used.

The impact of phosphite concentration on observed phytotoxicity symptoms on the two year old plants (from both the inoculation trial n = 42 and the phytotoxicity trial n = 30) was analysed with a binomial generalised linear model (logit link; package stats, version 4.2.3 (R Core Team 2023). There was no significant effect of the presence of Duwett® or trial type on the presence of phytotoxicity so these factors were combined in the analyses.

Pearson's chi-square with Yates continuity correction for any deviance from the Chi-square distribution was used to evaluate plant symptom expression (wilt scores), phosphite application timing (preventative vs curative), and Duwett® adjuvant effects (presence or absence) among treatment groups. Fisher's exact tests were used in place of Chi-squares where expected cell counts were < 5 (Agresti 2007; Fisher 1934).

Mosaic plots were used to show frequency data (wilt scores) for the *P. agathidicida* trunk inoculation assay of the 2 year old plants using the ggmosaic package (version 0.3.3) (Jeppson et al. 2021).

For the detached leaf assays from the 6-year-old plants, lesion areas were standardised to the lesion areas from the pre-phosphite treatment detached leaf assay for each plant to account for differences in leaf shape between plants.

Results

Inhibition of *Phytophthora agathidicida* colonisation and phytotoxicity impacts in 2-year-old kauri treated with foliar applied phosphite

Effect of adjuvant on wilt symptoms

There was no significant adjuvant effect on the presence of wilt symptoms in the plants treated with 7.5 g/L phosphite with or without Du-Wett® (Fisher exact test $p = 0.0564$, Fig. 1). Of the plants treated with Duwett® 42% had not wilted at day 18 DPI, compared with 83% of the plants treated with 7.5 g/L without Duwett® (Fig. 1).

Effect of phosphite application timing on wilt symptoms

The application of 7.5 g/l phosphite was more effective at reducing wilt symptoms when applied as a preventative treatment compared to its use as a curative treatment (Fishers exact test p-value = 0.009; Fig. 2). There was no significant difference in wilt scores for the preventative and curative treatments using 5 g/L phosphite (Fishers exact test p-value = 0.674; Fig. 2).

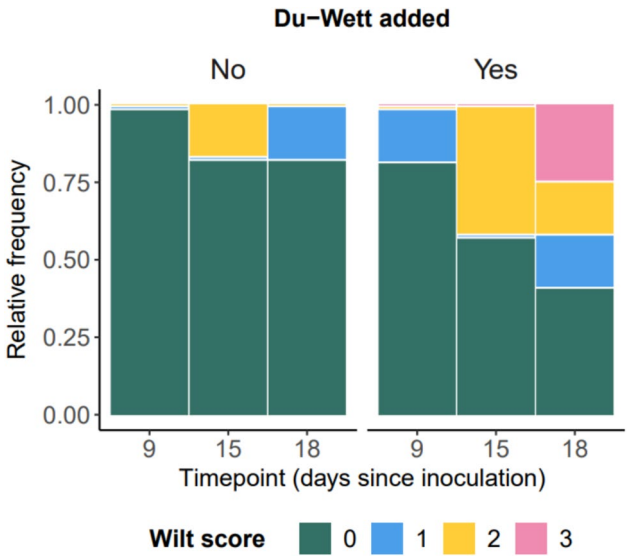


Fig. 1 Wilt scores for the 2-year-old kauri plants treated with 7.5 g/L phosphite with and without the addition of 0.2% Du-Wett® and inoculated with *Phytophthora agathidicida*. Green represents a wilt score of 0 = healthy, blue represents a wilt score of 1 = slight wilt, yellow represents a wilt score of 2 = moderate wilt, and pink represents a wilt score of 3 = severe wilt. Overall there was no significant adjuvant effect (Fisher exact test $p = 0.0564$)

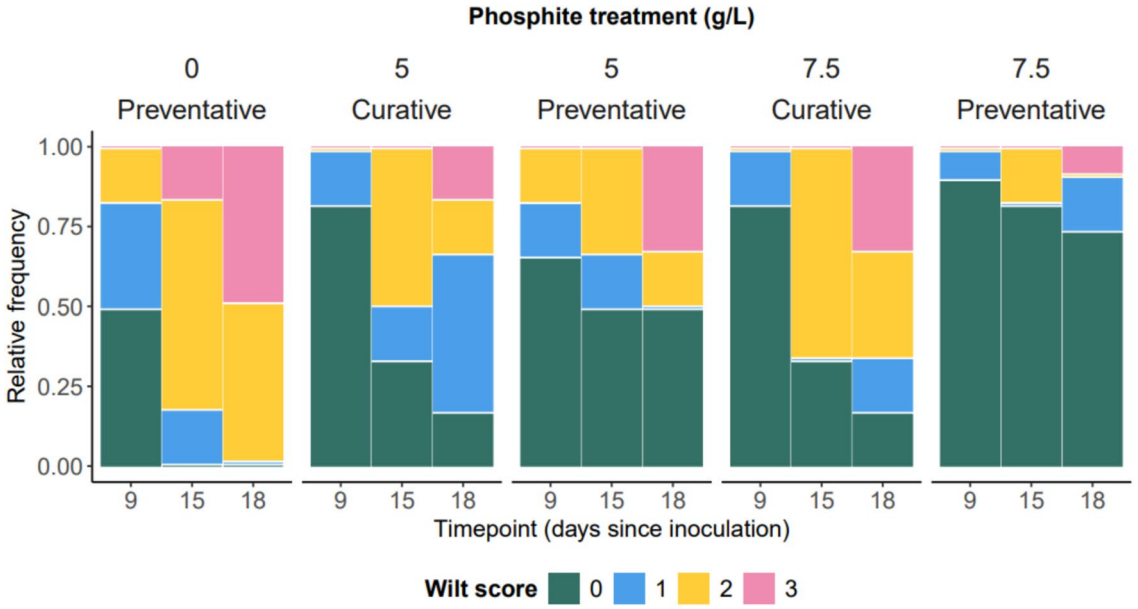


Fig. 2 Wilt scores for the *Phytophthora agathidicida* inoculated 2-year-old kauri plants treated with 0, 5 or 7.5 g/L phosphite as a curative or preventative treatment. Green represents a wilt score of 0 = healthy, blue represents a wilt score of 1 = slight wilt, yellow represents a wilt score of 2 = moderate wilt, and pink represents a wilt score of 3 = severe wilt

Inhibition of *P. agathidicida* infection and wilt symptoms

Phosphite treatments significantly reduced the colonisation of *P. agathidicida* in the 2-year-old plants as shown by a reduction in wilting in the 5 and 7.5 g/L treatments compared to the 0 g/L treatment (Fisher test $p = 0.$ 0.0009; Fig. 2). The inoculated trees treated with 0 g/L phosphite had all wilted after 18 days (scores ≥ 2; Fig. 2). None of the uninoculated control plants wilted (n = 6).

Phytotoxicity response

The likelihood of phytotoxicity in the < 1 year old leaves increased with increasing phosphite concentration (binomial glm; p-value = 0.007; Fig. 3). No phytotoxicity was observed on the old leaves > 1 year old. Phytotoxicity was observed in 67% of trees treated with 7.5 g/L + 0.2% Du-Wett®, and 42% of trees treated with 7.5 g/L without Du-Wett®. There was no significant effect of Duwett® on the presence of phytotoxicity in the 7.5 g/L phosphite treatments (chi square $p = 0.3282$). There was no difference in the presence of phytotoxicity between the inoculation trial and the phytotoxicity trials (chi-square $p = 0.162$).

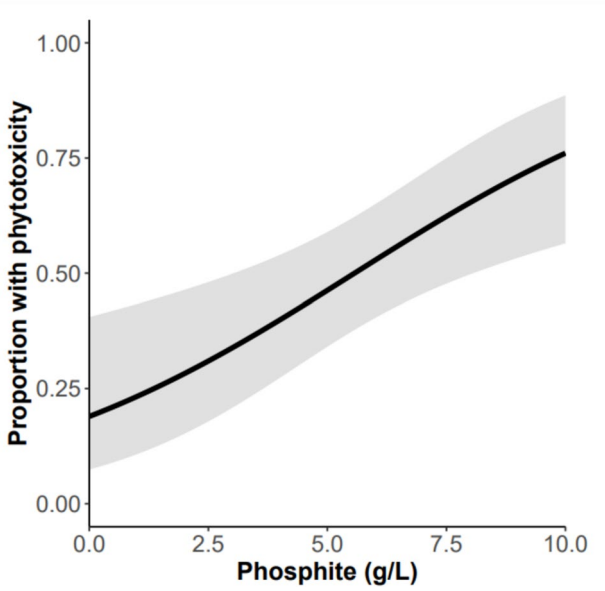


Fig. 3 The proportion of leaves with phytotoxicity symptoms from the current (< 1-year-old) flush in the 2-year-old kauri plants increased with increasing phosphite concentration. Data from the 2 year old kauri trial of *P. agathidicida* inoculation trial and the phytotoxicity monitoring trial were included. Plants were treated with Foschek® at 0, 5, 7,5 or 10 g/L with 0.2% Du-Wett®, or 7.5 g/L without Du-Wett®. Solid lines and surrounding grey areas indicate the fits and 95% confidence intervals (CI) from binomial generalised linear models (GLM) created in R with the package stats

Translocation of foliar applied phosphite in 6-year-old kauri and phytotoxicity response

Phytotoxicity response

Phytotoxicity symptoms were observed in the young (current flush) and older (≥ 1 year old) leaves for the 6-year-old kauri plants and the proportion of leaves with phytotoxicity increased with increasing phosphite concentration (Fig. 4A and B; binomial generalised linear model p-values = 0.0009 and 0.007, respectively). No phytotoxicity was observed on the trees treated with 0 g/L phosphite. The addition of Du-Wett® appears to reduce the incidence of phytotoxicity in the older leaves at the higher phosphite concentrations, though this was not statistically significant (Fig. 4B). The phytotoxicity symptoms in the older leaves were expressed as burn marks originating from the distal leaf drip tip.

Detached leaf bioassays from the 6-year-old kauri plants

Phosphite uptake in the foliage of the 6-year-old plants was assessed by inoculating detached leaves with *P. agathidicida* mycelium and measuring the area colonised (lesions). Phosphite treatments significantly reduced the lesion area produced in the detached leaf assays relative to the lesions on the control treatment (Wilcox p-values < 0.01; Fig. 5). Overall, there was no adjuvant effect on the percentage lesion area produced (Kruskal Wallis $p = 0.2878$). There was a significant difference in the percentage lesion area produced within the treatments between weeks 0 and 2 (Wilcox $p < 0.0001$) and 0 and 4 ($p < 0.0001$), respectively, but there was no difference in lesion size between weeks 2 and 4 (Wilcox $p = 0.5$; Fig. 5).

Root phosphite concentration

Eight weeks after the phosphite treatment, root samples were collected from the 6-year-old plants and sent for phosphite concentration testing. The results showed that the foliar application of phosphite was translocated to the roots of the plants, as the root phosphite concentration of the plants treated with 5 and 7.5 g/L phosphite was higher than the 0 g/L control treatment (Table 2).

Discussion

Foliar application of phosphite is widely used in the horticultural industry, production nurseries and natural ecosystems in Western Australia (Hardy et al. 2001), but phosphite foliar sprays are yet to be fully explored for the protection of kauri from infection by *P. agathidicida*. Phosphite-trunk injections are an important tool used to manage kauri dieback and are an efficient application method in easy-to-access forests; however, in difficult terrain and isolated stands of kauri, trunk injection becomes difficult and increases the risk of spreading soilborne *Phytophthora* inoculum. This study showed that foliar phosphite application may be a suitable method to protect kauri from *P. agathidicida*. In a glasshouse trial with 6-year-old kauri, it was demonstrated that

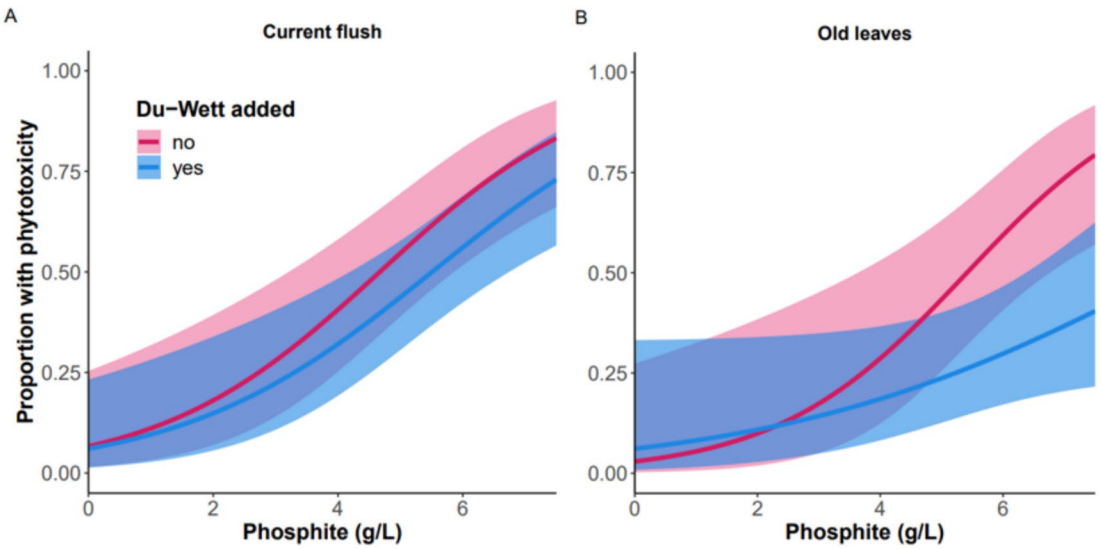


Fig. 4 The proportion of leaves with phytotoxicity symptoms from the **A**) current (< 1-year-old) flush and **B**) older (> 1-year-old) leaves in the 6-year-old kauri plants increased with increasing phosphite concentration. Plants were treated with Foschek® at 0, 5, 7,5 or 10 g/L with (blue) or without (pink) 0.2% Du-Wett®. Solid lines and surrounding shaded areas indicate the fits and 95% confidence intervals (CI) from binomial generalised linear models (GLM) created in R with the package stats

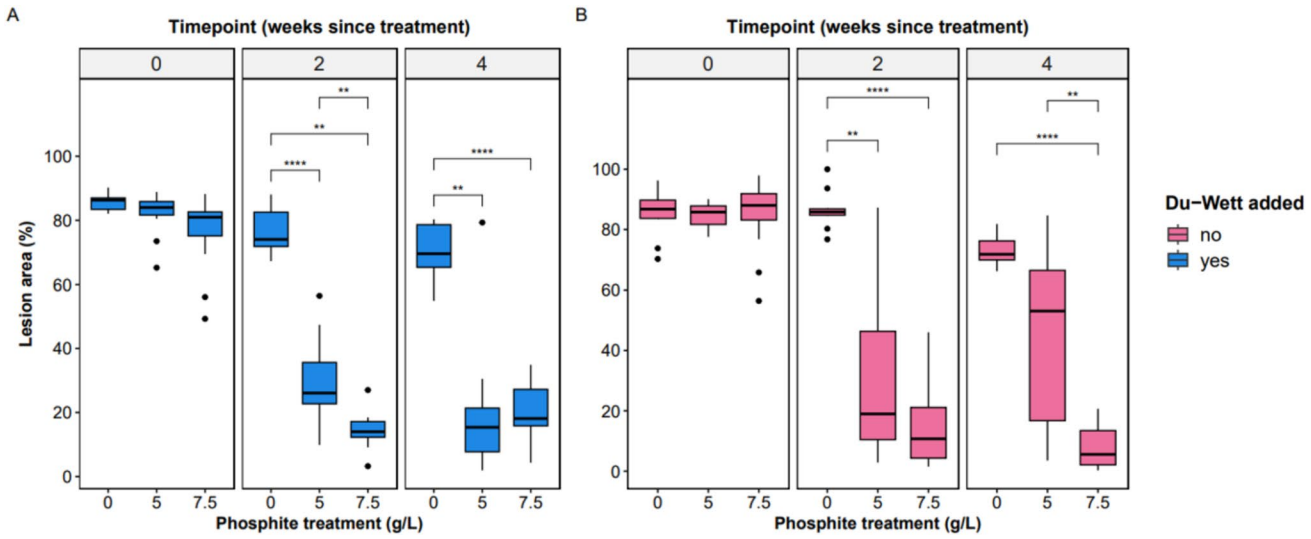


Fig. 5 Lesion area produced in a detached leaf bioassay on leaves taken from the 6-year-old kauri plants at 24 h prior to the phosphite treatment (timepoint 0) and 2- and 4-weeks post-treatment. The lesion area for the pre-phosphite timepoint (0 weeks) detached leaves was averaged. For the 2- and 4-weeks post-treatment time points the lesion area was calculated as relative to the lesion area from the pre-phosphite leaf assay to standardise for differences in leaf shapes and sizes across the trees. Significant pairwise Wilcox *p*-values are displayed (** represents p-values between 0.001 to 0.01, and **** represents *p*-values between 0.00001 and 0.0001). Panel **A**) is with the adjuvant Du-Wett® at 0.2% (blue), and panel **B**) is without Du-Wett® (pink)

Table 2 Results from the root phosphite concentration testing conducted by a third-party lab (Hills Laboratory Limited, Hamilton, New Zealand)

Sample	Phosphite treatment (g/L)*	Phosphorous acid concentration	
		mg/kg as received	mg/kg at 75% moisture
A	0	< 2	< 2
C	5	9	8
E	7.5	8	6

*all three treatments included Du-Wett® at 0.2%

phosphite is translocated to the root system and the colonisation of *P. agathidicida* was prevented in both sapling trunk inoculation and detached leaf bioassays.

Phytotoxicity

Phytotoxicity was consistently observed in the leaves from the current flush (< 1 year-old) in both glasshouse experiments. The symptoms appeared soon after application and no ill effects on foliage emerged after the treatment. Applications that avoid the spring flush could significantly reduce the impacts of phytotoxicity. Phytotoxicity was not observed on older leaves in the 2-year-old plants but was seen in the 6-year-old plants in those plants treated with 7.5 g/L and no Du-Wett®. The 6-year-old plants were sprayed to run off with a larger volume of treatment (100 mL) while the 2-year-old plants were sprayed to drip. It is not surprising that phytotoxicity was observed at the higher rate of 7.5 g/L as this is above the recommended 5 g/L rate for foliar application (Hardy et al. 2001). Kauri leaves take 8 months to mature and can remain on kauri trees for up to 15 years (Clarkson 1992; Silvester and Orchard 1999). So, if young leaves (< 1 year old) suffer from phytotoxicity following a foliar phosphite treatment, this cohort of leaves would remain affected for the duration of their life.

The two glasshouse trials in this study demonstrated that phosphite is effectively translocated in kauri when applied as a foliar spray across a range of plant ages and growth conditions. While higher treatment levels may induce minor phytotoxicity symptoms, these effects do not result in long-term adverse impacts on plant health.

Adjuvant effect

The use of Foschek® without the inclusion of Du-Wett® was slightly more effective at preventing wilting caused by the colonisation of *P. agathidicida* in the 2-year-old plants, though this was not statistically significant. In Western Australia the surfactant Synertrol Oil (Organic Crop Protectants Pty Ltd), is used (Hardy et al. 2001). Foschek® has a surfactant already included in the product, which is likely to have contributed to phosphite uptake and greater inhibition of *P. agathidicida* colonisation in the 2-year-old plants without the addition of Du-Wett®. The surfactant enhances

the spreading of the treatment and creates a thin film on the surface of the plant with the excess more readily dripped off. In the 6 year old plants, burning symptoms were observed originating from the distal leaf tip where the excess treatment accumulated and dripped from. Further work is needed to test if the addition of a surfactant improves the application of phosphite for kauri in a field setting.

Preventative treatment: phosphite barrier

The preventative application of phosphite prior to the infection of 2-year-old plants was more effective at limiting infection impacts compared to the curative treatment following infection at the 7.5 g/L phosphite concentration. This provides initial evidence that prophylactic phosphite applications to uninfected kauri trees inhibits the colonisation of *Phytophthora*. Preventative phosphite applications could be used to create a treatment barrier to prevent the movement of the pathogen from diseased into healthy forest areas (Crane and Shearer 2014; Horner 2016).

Considerations for field application of foliar sprays in kauri forests

There are several things to consider before using foliar application to treat kauri with phosphite in the field. Sensitive plants in the kauri ecosystem, including epiphytes commonly associated with kauri, should be screened for phytotoxicity to both phosphite and candidate adjuvants as some can cause phytotoxicity in their own right (Hardy et al. 2001). The longevity of foliar applications may not be as long-lasting as injections (Hardy et al. 2001; Shearer and Fairman 2007) so the long-term efficacy, re-treatment period and cost-efficacy need consideration. Kauri stomatal conductance is highest in the morning before 9 am (Macinnis-Ng et al. 2017) so this may be the best time to apply foliar sprays. Sap flow and stomatal conductance in kauri are highest in the summer and when trees are not in drought (Zhao et al. 2019).

Leaf morphology (stomata abundance and distribution, waxiness, leaf hairs, leaf shape) affects *in planta* concentrations of phosphite spray by influencing spray distribution, retention and uptake of the chemical by the plant (Barrett 2001; Shearer et al. 2012). The majority of kauri stomata (> 95%) are on the abaxial surface (Macinnis-Ng et al. 2017), so application should target this area. A low-volume spray with fine droplet size would be suitable (Maski and Durairaj 2010). While Foschek™ (400 g/L phosphorous acid present as mono-di potassium phosphite) contains proprietary surfactants, there may be opportunities to further optimise its formulation based on kauri leaf or stem physiology (Nairn and Forster 2021).

Future work

The logical next step of this research is to conduct controlled field trials with foliar-applied phosphite treatments. This research has shown that 5 g/L phosphite, the same rate used in Australia, should be appropriate for kauri as it is effective at limiting *P. agathidicida* colonisation, is translocated to the roots and has minimal phytotoxicity. Further investigation is needed to determine whether phosphite application impacts plant community composition through the suppression of oomycete activity or whether there are negative effects on the flora and fauna associated directly with kauri trees. While an initial study has indicated phytotoxicity in native plants is unlikely (Scott et al. 2016), this study did not investigate the impact on the diverse range of epiphytic plants commonly associated with kauri. There have been concerns of phosphite application altering soil phosphate (P) levels (due to oxidisation by microbes), as the soil around kauri is characteristically low in nutrients, including P. As a consequence, this is something to consider where there may be repeated applications to a given area over time (Verkaik and Braakhekke 2007). A recent study showed that phosphite had less of a negative effect on low P-adapted plants than expected (Boulle et al. 2023). Efficacy trials are required to determine the optimal phosphite concentration and retreatment frequency needed to maintain control using foliar phosphite applications. This important work is also yet to be done for trunk injection application. Phosphite injections in kauri appear to last for 10 years or more (Horner 2020; Horner et al. 2024), but we do not know what the latency period is between treatment, re-infection and then symptom expression.

Additives, such as salicylic acid, synthetic elicitors or calcium (Stasikowski et al. 2014) could induce plant defence responses and improve the efficacy of single treatments. These may increase the longevity of treatments, reducing reapplication rates, costs and adverse environmental effects. Synthetic elicitors can induce plant immune responses, provide protection from pathogens and boost the health of already infected hosts (Bektas and Eulgem 2015). Synthetic elicitors can be applied as foliar sprays and have been trialled with many horticultural pathogens (Bektas and Eulgem 2015). Benzothiadiazole (BTH), a synthetic analogue of salicyclic acid, was shown to induce resistance to a range of pathogens including *Phytophthora sojae* in soybean seedlings (Han et al. 2013), *P. palmivora* in papaya seedlings (Zhu et al. 2002) and *P. parasitica* in tobacco (Friedrich et al. 1996). The protection provided by single phosphite treatments could be enhanced by the addition of synthetic elicitors or salicylic acid (Groves et al. 2015). Similarly, the combination of foliar application of phosphite with calcium sulphate to soil has been shown to significantly increase the efficacy of phosphite in controlling the development and

spread of *Phytophthora* dieback disease (Stasikowski et al. 2014). Such amendments may also allow for lower phosphite concentrations to be used for sensitive plants to avoid phytotoxic responses.

Conclusions

Phosphite is an effective tool to protect kauri from *P. agathidicida*. The current trunk injection application method, while effective, has its limitations and, most importantly, presents a risk of spreading the pathogen (by the technicians). This study has shown that foliar phosphite application inhibited *P. agathidicida* colonisation and that phosphite was translocated to the roots of treated trees. The next steps should involve phytotoxicity screening of sensitive epiphytes and controlled field trials to optimise the foliar application. Foliar treatment of kauri could be used in conjunction with trunk injection to protect isolated stands of kauri and minimise the spread of the pathogen from tree stands where the pathogen is established.

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Data availability The data will be made available upon request.

Declarations

Ethical Approval Not Applicable.

Conflicts of interest The authors declare there are no conflicts of interest.

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