Journal of Plant Pathology
https://doi.org/10.1007/s42161-025-01945-8

ORIGINAL ARTICLE

Evaluation of phosphite to protect a South African Proteaceae from Phytophthora root rot

Dumsani Msweli[1,2] · **Sjirk Geerts**[2] · **Mashudu Nndanduleni**[1] · **Trudy Paap**[3]

Received: 10 October 2024 / Accepted: 1 June 2025

Abstract

Phytophthora cinnamomi is a globally recognised invasive plant pathogen, affecting approximately 5000 host species. In South Africa, previous research has linked *P. cinnamomi* to root rot in endemic Proteaceae, including *Leucadendron argenteum*. Recent observations have noted high mortality rates in *L. argenteum*, with *P. cinnamomi* readily isolated from the roots and collars of dying trees. Phosphite is commonly used to control *Phytophthora* diseases, but its efficacy in protecting native South African flora remains uncertain. To address this, trials were conducted to evaluate phosphite's effectiveness against *P. cinnamomi* infection in *L. argenteum*. In the glasshouse trial, four-month-old seedlings were treated with 5 g/L phosphite and then inoculated with *P. cinnamomi*. Non-treated inoculated seedlings exhibited rapid wilting within three weeks, while treated seedlings showed significant disease reduction, with no difference in root weight and seedling height compared to non-inoculated controls. In the field trial, *L. argenteum* trees treated with 40 g/L and 50 g/L phosphite injections over 30 months showed no significant difference in survival rates compared to untreated controls. The lack of phosphite efficacy in the field trial was attributed to the presence of *Armillaria* spp. The study underscores the threat *P. cinnamomi* poses to *L. argenteum* populations and highlights the additional risk from *Armillaria*. While phosphite has demonstrated effectiveness against Phytophthora root rot, further investigation is needed to determine if *P. cinnamomi* and *Armillaria* spp. have synergistic effects on *L. argenteum* mortality. Additionally, exploring phosphite's potential to protect plants from *A. mellea* at the seedling stage is warranted.

Keywords *Armillaria* · Fynbos · Invasive pathogen · *Leucadendron argenteum* · Mediterranean ecosystem · *Phytophthora cinnamomi*

Introduction

The introduction of alien pathogens can have profound and detrimental effects on native ecosystems, disrupting ecological balance and threatening biodiversity (Tobin 2015). The oomycete genus *Phytophthora* contains many important plant pathogens, of which *P. cinnamomi* has a substantial impact (Summerell and Liew 2020). *Phytophthora cinnamomi* infection can cause root and collar rot and cankers in many plant species, which leads to dieback and death of the host plants (Hardham and Blackman 2018). *Phytophthora cinnamomi* has devastated ornamental, horticultural, and commercial forestry plants, as well as natural ecosystems, in numerous countries across the globe (Shearer and Fairman et al. 2007; Linde et al. 1997; Wilkinson et al. 2001; Hardham and Blackman 2018; Engelbrecht et al. 2022). As a result of its widespread impact, *P. cinnamomi* has been listed among the world's 100 worst invasive alien species, one of only three invasive forest pathogens on this list (Global Invasive Species Database 2024).

Approximately 5000 plant species around the world are known to be hosts of *P. cinnamomi* (Hardham and Blackman 2018), with Proteaceae amongst the most susceptible families (Shearer et al. 2013). While the exact origin of *P.*

✉ Dumsani Msweli
thamsweli@gmail.com

1 South African National Biodiversity Institute, Kirstenbosch National Botanical Gardens, Claremont, Cape Town 7735, South Africa

2 Department of Conservation and Marine Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa

3 Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa

Published online: 01 July 2025

cinnamomi remains uncertain, it was first described from Sumatra (Rands 1922), and evidence suggests a Southeast Asian origin (Engelbrecht et al. 2022). *Phytophthora cinnamomi* has a particularly high impact in Mediterranean-type ecosystems (Burgess et al. 2017). Notably, an estimated 40% of plant species in the South-West Australian Floristic Region (SWAFR) are susceptible to *P. cinnamomi* (Shearer and Fairman 2007; Cahill et al. 2008), with the pathogen considered to be one of the most significant threats to the conservation of the floral diversity of the region (Barrett and Rathbone 2018). Another Mediterranean region, the Cape Floristic Region (CFR) of South Africa, like the SWAFR, is rich in Proteaceae (Goldblatt 1997). Despite early evidence of *P. cinnamomi* impact on South African Proteaceae (van Wyk 1973; von Broembsen 1984), the impact of *P. cinnamomi* on the CFR flora is still largely unexplored. However, it is known that *P. cinnamomi* decimates susceptible Australian Proteaceae planted in the CFR for floriculture (Qongqo et al. 2022). More recent observations by Paap et al. (2023) suggest that research on the impacts and control of *P. cinnamomi* on native Proteaceae is long overdue.

Leucadendron argenteum (L.) R.Br. (Proteaceae) is an iconic tree of the CFR and endemic to the Cape Peninsula of South Africa (Heelemann et al. 2013). Classified as "Vulnerable" in the Red List of South African Plants (Rebelo et al. 2020), this species has lost 55% of its habitat due to agriculture and urbanisation. Threats include altered fire regimes, alien plant invasion, predation of seeds by alien squirrels, and susceptibility to root fungal infections (Rebelo et al. 2020). While *P. cinnamomi* is not considered a major threat currently (Rebelo et al. 2020), there is evidence that it should be (van Wyk 1973; von Broembsen 1984; Heelemann et al. 2013; Hulbert et al. 2019). *Phytophthora cinnamomi* has impacted *L. argenteum* populations since the 1940s; however, it was thought to be bark and wood boring beetles or the fungus *Botryosphaeria ribis* (now *Neofusicoccum ribis)* that were responsible for the sudden deaths of many individuals (Olivier 1951). It was only in the early 1970s that van Wyk (1973) diagnosed *P. cinnamomi* as the cause of the sudden death in *L. argenteum* plants. According to Coetzee et al. (2018), *P. cinnamomi* frequently infects fynbos species in the Kirstenbosch National Botanical Garden, and Hulbert et al. (2019) confirmed the widespread presence of *P. cinnamomi* across this garden.

Phytophthora cinnamomi can spread to new areas by drainage water runoff, infected soil adhering to wheels, boots, work equipment, and plant material (Summerell and Liew 2020). Once established, eradication is nearly impossible (Dunstan et al. 2010). Until the late 1980s, when phosphite was introduced, the primary technique for controlling *P. cinnamomi* in natural ecosystems was to map the infected areas and rely on hygienic practices to prevent further disease spread (Wilkinson et al. 2001).

A reduced form of phosphate, phosphorous acid, or phosphite, is a systemic fungicide and is the primary method of chemical control for *Phytophthora* diseases in agriculture, forestry, and natural ecosystems globally (Wilkinson et al. 2001; Graham 2011; Akinsanmi and Drenth 2013; Tkaczyk et al. 2016). The mode of action is to induce the plant's defence mechanism rather than a direct antimicrobial action (Garbelotto et al. 2007). Phosphite protects the plant by increasing the thickness of the cell wall, enhancing lignification, and enhancing secondary plant metabolite production, which stimulates the defence of the host plant (Wilkinson et al. 2001; Garbelotto et al. 2007). It was first reported in 1989 that phosphite could suppress *P. cinnamomi* colonisation in *Banksia grandis*, a SWAFR plant species (Wilkinson et al. 2001). Barrett and Rathbone (2018) demonstrated that phosphite remains effective after repeated use, with no negative effects on the environment or pathogen resistance. In South Africa, phosphite is currently used to protect agricultural crops from *P. cinnamomi* (Masikane et al. 2020), but whether it can reduce mortality in native taxa, such as Proteaceae, is largely unknown.

This study aimed to (i) assess the effectiveness of phosphite foliar application to control *P. cinnamomi* infection and disease development in *L. argenteum* under glasshouse conditions and (ii) evaluate the effectiveness of phosphite stem injections in mature *L. argenteum* trees in a field experiment.

Materials and methods

Study site

This study took place in a natural area within the Kirstenbosch National Botanical Garden (KNBG) mother stock area (−33.99264, 18.42866), silver tree avenue (−33.99428, 18.42419), and the research glasshouse (−33.98514, 18.43656). Part of KNBG consists of natural vegetation, and the *L. argenteum* trees in the mother stock area occur naturally, unlike those that were planted in 2013 in the adjacent silver tree avenue, for the purpose of ex-situ conservation.

Glasshouse trial

Seed propagation and transplanting of seedlings

The seeds of *L. argenteum* were collected from the KBNG mother stock area and cleaned by removing them from the cone and soaking them in a 0.144 g/L hydrogen peroxide solution overnight to induce the opening of capsules. The *L.*

argenteum seeds were exposed to fynbos smoke to mimic the natural smoke scarification of the fynbos. For smoke treatment, the fire was started in a drum containing wet and dry fynbos plant material, and the smoke was directed into a polythene tent where seeds were placed, using the technique outlined in de Lange and Boucher (1990). Thereafter, the seeds were sown into polystyrene trays containing a fynbos medium for germination, consisting of 70% sand and 30% fine bark. All trays were irrigated three times a week. After germination, seedlings were planted into 165-mm-diameter free-draining pots filled with washed coarse river sand. Two sterile polyurethane inoculation tubes (150 mm long, 20 mm diameter) were placed in each pot at the time of transplanting. Seedlings were placed under a 50% shade net and irrigated daily using overhead sprinklers. Seedlings were fertilised twice a week with Seagro™ organic fertiliser, following the manufacturer's instructions. Two weeks prior to inoculation, the seedlings were relocated to a naturally ventilated glasshouse with a temperature range of 10.5–38.5 °C. Seedlings were irrigated with a drip system that ran for 5 minutes every day and fed with Seagro™ organic fertiliser twice a week.

Inoculum preparation

The *P. cinnamomi* isolate used in the current study was obtained from a collar lesion of a *L. argenteum* tree in the KNBG mother stock area (−33.99268, 18.42865). The isolate was obtained by the direct plating of collar pieces on to *Phytophthora* selective media, as described by Paap et al. (2023), with morphological identification based on the presence of coralloid hyphae with hyphal swellings, a characteristic typical of *P. cinnamomi*. This identification was confirmed by sequencing the internal transcribed spacer regions 1 and 2 (ITS), including the 5.8S rRNA region, using the primers ITS6 (Cooke et al. 2000) and ITS4 (White et al. 1990), as described by Paap et al. (2023). The isolate was deposited in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (CMW 58,748) with the sequence deposited in GenBank (accession number PP327027). The inoculum was prepared following the method of Bose et al. (2019). In brief, 500-ml Erlenmeyer flasks, each containing 250 ml of vermiculite (Bark Unlimited Organics), 5 g millet seeds Westerman™, and 300 ml of 10% clarified V8 juice (Campbell Soup Company) were plugged with non-absorbent cotton wool and autoclaved for three consecutive days. Upon cooling, the flasks were inoculated with agar blocks of the *P. cinnamomi* isolate (10 × 10 mm), which had been grown on half strength Potato Dextrose Agar (1/2 PDA; 19.5 g PDA powder, Merck, South Africa, 7 g Difco™ agar, 1 L deionised water) for 6 days. Thereafter, the flasks were incubated at 20 °C. Flasks were slightly shaken to distribute the inoculum evenly. After 6 weeks of incubation, the inocula were rinsed using sterilised, deionised water to wash off excessive nutrients.

Experimental design

A total of 80 four-month-old *L. argenteum* seedlings were used in a controlled glasshouse trial to evaluate the efficacy of phosphite in controlling *P. cinnamomi*-induced disease. The experiment followed a factorial design with phosphite treatment and pathogen inoculation as the two main factors, resulting in four treatment groups with 20 replicates each: (i) phosphite-treated and inoculated, (ii) phosphite-treated and non-inoculated, (iii) non-treated and inoculated, and (iv) non-treated and non-inoculated controls.

Phosguard 400 SL (400 g/L phosphorous acid equivalent) was diluted to a final concentration of 5 g/L, mixed with a wetting agent (Ecoguard Actipron Super) according to the manufacturer's instructions, and applied to the foliage using a knapsack sprayer until there was run-off from the leaves. For the following 48 hours, seedlings were irrigated manually on the roots to prevent phosphite from washing off. Two weeks later, half of the phosphite-treated and non-treated seedlings were inoculated with *P. cinnamomi* by removing the inoculation tubes and introducing 2.5 g of inoculum into each cavity. Non-inoculated controls received an equal amount of sterile vermiculite medium. The cavities were then sealed with sterile, coarse river sand. The trial was harvested three weeks after inoculation, at which point the untreated inoculated seedlings had started to wilt, indicating the initial stages of seedling mortality.

Measurement of symptoms

The response of the seedlings to the four treatments were evaluated by measuring changes in seedling height and assessing the severity of root rot. Seedling height was measured at the time of inoculation and again at harvest, to determine the extent to which the treatments influenced seedling growth. At the time of harvest, roots were carefully washed under running water to remove sand, ensuring minimal damage. Root rot severity was visually rated on a scale from 0 to 4, where 0 indicated no visible root damage, 1 represented approximately 20% of the roots exhibiting lesions and loss of fine roots, 2 corresponded to more than 20% but less than 50% of the roots affected, 3 indicated over 50% of the roots with lesions and fine root loss, and 4 signified complete root rot and seedling death. To further evaluate treatment effects, shoots and roots were separated at the stem base, and the roots were placed in paper bags and dried in an oven at 40 °C for two weeks before being weighed.

Re-isolation and identification of *P. cinnamomi*

To confirm Koch's postulates, a small piece of root from each seedling was plated onto *Phytophthora*-selective medium (NARPH; 50 mg nystatin, 200 mg ampicillin, 10 mg rifampicin, 25 mg pentachloronitrobenzene and 50 mg hymexazol per 1 L of deionised water and 17 g cornmeal agar). Growth of the pathogen was confirmed by microscopy based on the presence of coralloid hyphae with hyphal swellings, characteristic of *P. cinnamomi*.

Field trial

Experimental design

At the mother stock section of the KNBG, 50 naturally occurring *L. argenteum* trees with diameters at breast height (DBH) of 5.3–15.8 cm [mean (± SD) of 9.06 ± 2.87 cm] underwent stem injection with a 40 g/L phosphite solution, while 50 control trees with DBH of 3.9–15.2 cm (8.45 ± 2.78 cm) were left untreated. Similarly, at the planted silver tree avenue, fifty 7-year-old *L. argenteum* trees with DBH of 6.1–16.2 cm (10.20 ± 2.24 cm) were injected with a 50 g/L phosphite solution, while fifty control trees with DBH of 4.7–15.5 cm (9.68 ± 2.83 cm) were designated as controls. The control trees did not receive any treatment as drilling may have caused unnecessary harm to an already vulnerable species. Treated trees were distinguished by red spray paint markings, while control trees were marked in green. The presence of *P. cinnamomi* within the field trial area was previously confirmed by Hulbert et al. (2019), as well as by sampling of symptomatic trees during the current study as detailed in subsection 2.2.2.

Injection

The treated trees were first injected in December 2020. The holes were drilled through the cambium using a 4 mm drill bit, at the breast height level. The holes were drilled every 15 cm around the trunk circumference, delivering 0.8 g ai per 15 cm for mother stock trees and 1 g ai per 15 cm for silver tree avenue trees. Injectors (20 mL, spring-loaded, Chemjet, Bongaree, Qld, Australia) containing 40 g/L (0.05 g/cm) and 50 g/L (0.06 g/cm) phosphite (Phosguard 400 SL) were screwed into the holes and left until the tree had sucked up all the content from the injectors. Approximately 20 minutes were required to empty each injector. Eighteen months later, in May 2022, a second round of phosphite treatment was done on the surviving trees that were previously treated. The trees were scored as dead/alive every 6 months over a two-and-a-half-year period.

Identification of the cause of death of experimental trees

At the end of the trial, *P. cinnamomi* was isolated from the collar lesion of a non-treated tree that had died within the last 6 months i.e., the tree had still been alive at the previous assessment time point. During this sampling, white mycelial mats typical of those produced by *Armillaria* species were observed on dead trees. A representative sample was taken for species identification. This was confirmed by sequencing of the ITS region of the rDNA (ITS1–5.8S–ITS2) using the primers ITS1 and ITS4 (White et al. 1990), as described by Tchotet Tchoumi et al. (2017).

Statistical analyses

The glasshouse and field trial datasets were analysed using R v4.2.3 (R Core Team 2023), with the *FSA* (Ogle 2023), *rcompanion* (Mangiafico 2024), and *ggplot2* (Wickham 2016) packages.

Glasshouse trial

Before analyses, seedling height and dry root weight data were tested for normality using a Shapiro-Wilks (1965) test. A Kruskal-Wallis (1952) test was used for non-parametric data, with a one-way Analysis of Variance (ANOVA; Girden 1992) used for parametric data. Post hoc tests were used for pairwise comparisons between treatments for factors that proved significant after the main tests (Tukey HSD tests for parametric data and Dunn's test for nonparametric data).

To exclude confounding effects, seedling height at the time of inoculation was compared between the treatments. The data were non-parametric ($W = 0.965$, $P = 0.0278$), therefore, seedling height data were compared using the Kruskal-Wallis test. Seedling height data at harvest time were parametric ($W = 0.985$, $P = 0.456$) and therefore analysed using ANOVA with a Tukey HSD post hoc test.

Comparisons of dry root weight between the treatments were conducted using the Kruskal-Wallis test due to the non-normal distribution of the data ($W = 0.762$, $P < 0.001$). Post hoc analysis was conducted using Dunn's test with Bonferroni correction for multiple comparisons.

The association between treatment and root health score was assessed using a Fisher's exact test for count data (a non-parametric test suitable for analysing categorical data with small sample sizes). Pairwise comparisons between treatment groups were performed using the Fisher's exact test to assess the association between treatment types and root health status. Given the multiple comparisons, a Bonferroni correction was applied.

Table 1 Mean and standard errors (SE) of *Leucadendron argenteum* seedling height at inoculation and harvest, and dry root weight, for plants under different treatments. The treatment groups are denoted as follows: -Phi-Pc (control), + Phi + Pc (phosphite-treated and *Phytophthora cinnamomi* inoculated), + Phi-Pc (phosphite-treated only), and -Phi + Pc (*P. cinnamomi* inoculated only)

Treatment	Mean height at inoculation (cm)	SE	Mean height at harvest (cm)	SE	Mean dry root weight (g)	SE
+Phi–Pc	12.53	0.63	17.28	0.82	1.72	0.34
+Phi + Pc	12.63	0.68	15.73	0.82	1.05	0.23
–Phi + Pc	13.00	0.72	14.95	0.88	0.33	0.05
–Phi–Pc	12.90	0.70	18.20	0.90	1.07	0.13

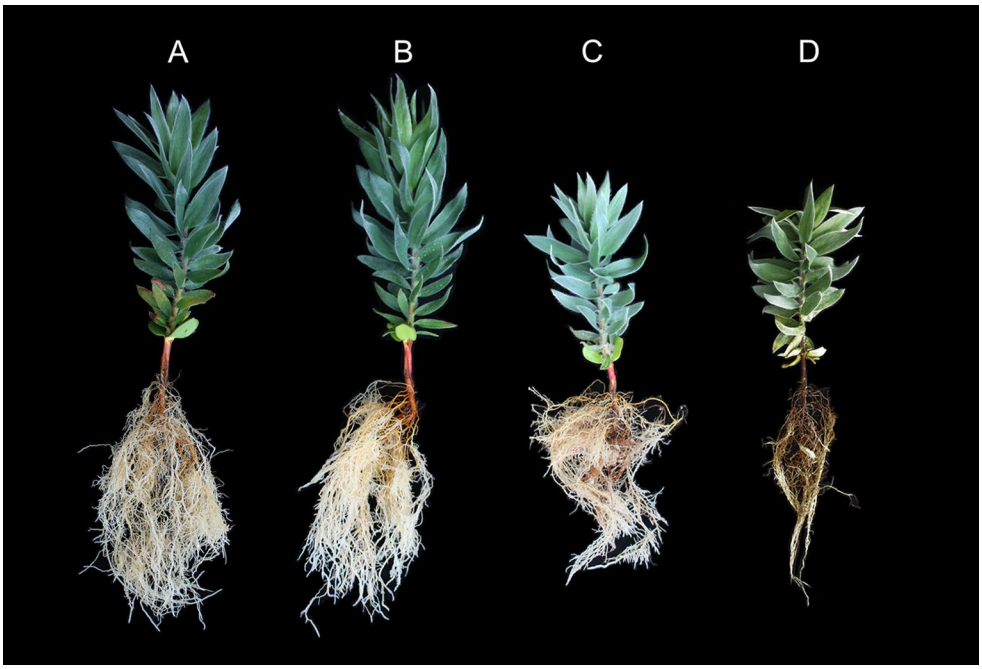

Fig. 1 The effect of phosphite treatment on disease development such as root rot and wilting caused by *Phytophthora cinnamomi* in *Leucadendron argenteum*. (**a**) treated non-inoculated control, (**b**) non-phosphite treated non-inoculated control, (**c**) phosphite treated *Phytophthora cinnamomi* inoculated and (**d**) non-phosphite treated and *Phytophthora cinnamomi* inoculated

Field trial

For the injection trial mortality data, only the last monitoring time point (30 months) was used. Data were analysed using a generalised linear model (GLM) with treatment (phosphite/control), site (mother stock and silver tree avenue) and their interactions as factors, and DBH as a continuous variable. Tree survival was modelled as a binary response variable, where '0' represents survival and '1' represents mortality. The GLM was specified with a binomial family and a complementary log-log link function.

Results

Glasshouse trial

Seedling height

At the time of inoculation, there was no significant difference in seedling heights between treatments ($\chi^2 = 0.26$, df = 3, P = 0.97) (Table 1). Within three weeks after inoculation, non-treated inoculated seedlings began to wilt (Fig. 1).

Table 2 Results of the Tukey HSD post hoc test comparing *Leucadendron argenteum* seedling height among four treatment groups at harvest, showing the difference in means, the corresponding confidence intervals (CI), and the p-values for each comparison. The treatment groups are denoted as follows: -Phi-Pc (control), + Phi + Pc (phosphite-treated and *Phytophthora cinnamomi* inoculated), + Phi-Pc (phosphite-treated only), and -Phi + Pc (*P. cinnamomi* inoculated only)

Comparison	Difference	Lower CI	Upper CI	p-value
–Phi + Pc vs –Phi–Pc	−3.497	−6.706	−0.289	0.027
+Phi–Pc vs –Phi–Pc	−1.172	−4.381	2.036	0.772
+Phi + Pc vs –Phi–Pc	−2.722	−5.931	0.486	0.125
+Phi–Pc vs –Phi + Pc	2.325	−0.842	5.492	0.225
+Phi + Pc vs –Phi + Pc	0.775	−2.392	3.942	0.918
+Phi + Pc vs + Phi -Pc	−1.550	−4.717	1.617	0.575

There was a significant effect of treatment on seedling height at harvest (F = 3.297, df = 3, P = 0.025), with inoculated seedlings significantly shorter than the non-inoculated seedlings (P = 0.027) (Table 1 and 2; Figs. 1 and 2).

Dry root weight

There were significant differences in dry root weight of seedlings from different treatments ($\chi^2 = 28.407$, df = 3, P < 0.001) (Figs. 1 and 3). The dry root weight of seedlings only treated

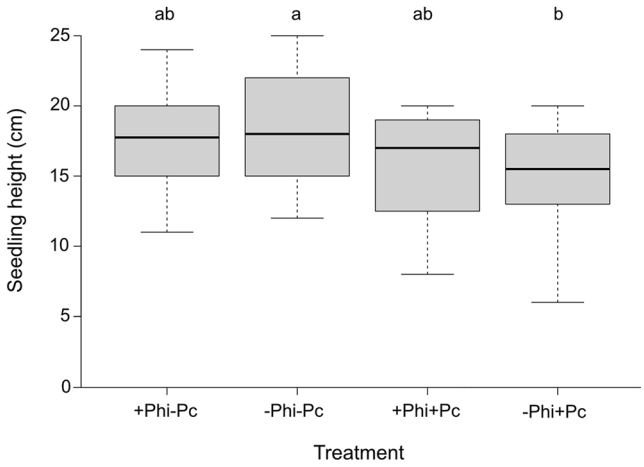

Fig. 2 Height of *Leucadendron argenteum* seedlings at harvest, for phosphite treated without *Phytophthora cinnamomi* inoculation (+Phi-Pc), non-treated and non-inoculated seedlings (-Phi-Pc), phosphite treated and inoculated seedlings (+Phi + Pc), and seedlings that were only inoculated (-Phi + Pc). N = 20 for each treatment. The horizontal line in the box is the median; the upper and lower boundaries of the box represent the interquartile range, and whiskers extend from maximum to minimum

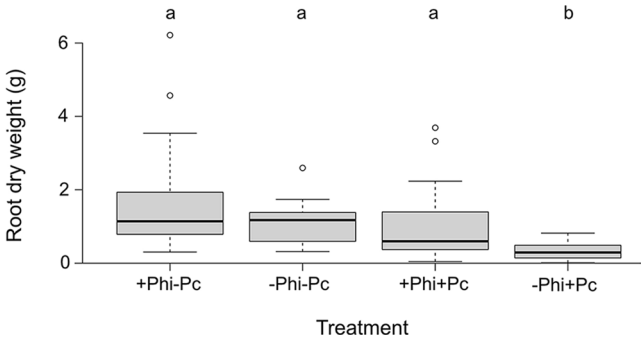

Fig. 3 The root dry weight of *Leucadendron argenteum* seedlings at harvest, for phosphite treated without *Phytophthora cinnamomi* inoculation (+Phi-Pc), non-treated and non-inoculated seedlings (-Phi-Pc), phosphite treated and inoculated seedlings (+Phi+Pc), and seedlings that were only inoculated (-Phi+Pc). N=20 for each treatment. The horizontal line in the box is the median; the upper and lower boundaries of the box represent the interquartile range, and whiskers extend from maximum to minimum

Table 3 The pairwise comparisons between different treatments for *Leucadendron argenteum* dry root weight. Adjusted p-values are corrected for multiple comparisons using the Bonferroni method. The treatment groups are denoted as follows: -Phi-Pc (control), +Phi+Pc (phosphite-treated and *Phytophthora cinnamomi* inoculated), +Phi-Pc (phosphite-treated only), and -Phi+Pc (*P. cinnamomi* inoculated only)

Comparison	Z Value	Unadjusted p-value	Adjusted p-value
–Phi–Pc vs–Phi + Pc	4.068	<0.0001	0.0002
–Phi–Pc vs + Phi–Pc	-0.870	0.39	1.0
–Phi + Pc vs + Phi–Pc	-5.002	<0.0001	<0.0001
–Phi–Pc vs + Phi + Pc	1.157	0.24	1.0
–Phi + Pc vs + Phi + Pc	-2.949	0.003	0.019
+Phi–Pc vs + Phi + Pc	2.053	0.04	0.24

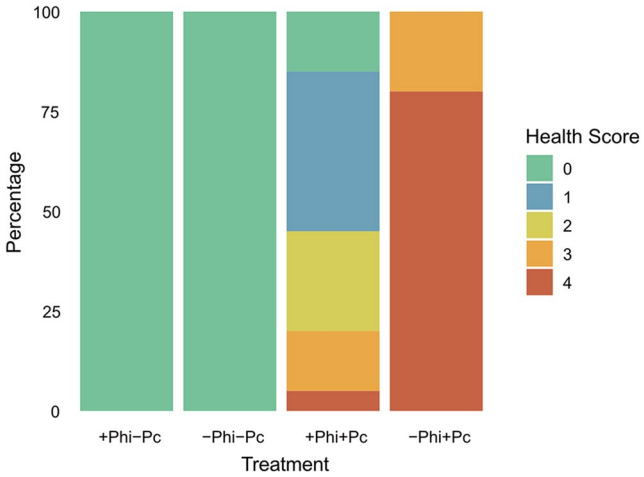

Fig. 4 Root health score for the four different treatments: seedlings only treated with phosphite (+Phi-Pc), seedlings not treated and uninoculated (-Phi-Pc), seedlings treated with phosphite and inoculated with *P. cinnamomi* (+Phi + Pc), and seedlings just inoculated with *P. cinnamomi* (-Phi + Pc). The Y-axis displays the percentage of plants (n = 20 per treatment) that have a specific health score for each treatment. Green indicates no visible root damage, and red indicates that roots have died

Table 4 Pairwise comparison of treatments for Bonferroni-corrected p-values for root health score outcomes of *Leucadendron argenteum* seedlings. The treatment groups are denoted as follows: -Phi-Pc (control), + Phi + Pc (phosphite-treated and *Phytophthora cinnamomi* inoculated), + Phi-Pc (phosphite-treated only), and -Phi + Pc (*P. cinnamomi* inoculated only)

Treatment comparison	Bonferroni-corrected p-value
–Phi–Pc vs + Phi + Pc	<0.0001
–Phi–Pc vs + Phi–Pc	N/A
–Phi–Pc vs + Phi + Pc	<0.0001
–Phi + Pc vs + Phi–Pc	<0.0001
–Phi + Pc vs + Phi + Pc	<0.0001
+Phi–Pc vs + Phi + Pc	<0.0001

with phosphite was 1.72 ± 0.34 g (mean ± SE), phosphite and inoculated 1.05 ± 0.23 g, only inoculated 0.33, ± 0.05 g and the control group had a dry root weight of 1.07 ± 0.13 g (Table 1). There was no significant difference in dry root weight between the treated and non-treated non-inoculated controls and treated inoculated seedlings; however, the non-treated inoculated seedlings had significantly lower dry root weight than all other treatments (Fig. 3; Table 3).

Root damage score

No root damage was observed for the non-inoculated seedlings (Figs. 1 and 4). A significant difference in root health scores was observed between all pairs of treatment groups, except for the two non-inoculated groups, where all seedlings in these treatments had health scores of 0 (no visible root damage) ($p < 0.001$; Table 4; Fig. 4). The severity of

root rot was the highest in non-treated inoculated seedlings, with all seedlings exhibiting either loss of fine roots and > 50% of roots with lesions or death of all roots. The severity of root rot in treated inoculated seedlings was significantly less than that observed in non-treated inoculated seedlings (Table 4; Figs. 1 and 4). While some loss of fine roots and root lesions were observed in some of the treated inoculated seedlings, healthy new root tips continued to be produced in all but one of the treated inoculated seedlings (Fig. 4).

Re-isolation of *Phytophthora cinnamomi*

Phytophthora cinnamomi was not isolated from any of the non-inoculated seedlings. It was, however, isolated from the roots of all inoculated seedlings.

Field trial

In total, 39 of the 50 trees treated with 40 g/L phosphite survived in the planted population, compared to 37 out of the 50 control trees (Fig. 5). In the natural population, 28 trees treated with 50 g/L phosphite survived, compared to 31 control trees (Fig. 5). Phosphite treatment did not impact tree survival, with none of the predictors, including site/treatment combinations and diameter at breast height (DBH), being significant (P > 0.05 for all analyses) (Table 5). Collar lesions indicative of *P. cinnamomi* infection were observed on dying and recently dead trees (Fig. 6). Additionally, *Armillaria* mycelial mats were observed on dead treated and untreated trees (Fig. 6), with an isolate of *A. gallica* obtained from a dead treated tree. The identity of *A. gallica* was confirmed by sequencing and the isolate deposited in the FABI culture collection (CMW 66,191, GenBank accession number PV460207).

Discussion

The current study demonstrates that when *L. argenteum* seedlings are inoculated with *P. cinnamomi,* it rapidly induces root rot, leading to wilting within three weeks. However, root damage was significantly reduced, and root weight was significantly higher in seedlings treated with phosphite. While loss of fine roots and root lesions were observed in some treated inoculated seedlings, healthy new fine roots were also observed. These results highlight the susceptibility of *L. argenteum* to *P. cinnamomi,* but importantly, they also show the efficacy of a single foliar application of 5 g/L phosphite in protecting *L. argenteum* seedlings from *P. cinnamomi* infection (Fig. 1).

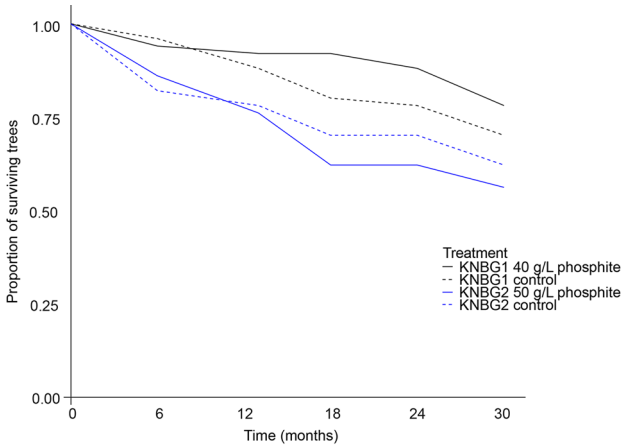

Fig. 5 The survival rate of *Leucadendron argenteum* trees (n = 200) over six-month intervals for 30 months. Trees treated with 40 g/L phosphite at KNBG1 (mother stock area) and those treated with 50 g/L at KNBG2 (silver tree avenue) are indicated by solid lines, while control trees are depicted with dotted lines. Two sites were monitored, one planted (KNBG2) and one natural (KNBG1). Both of these sites are infested with *Phytophthora cinnamomi*

Table 5 The estimated coefficients, standard errors, z-values, and p-values for predictors of *Leucadendron argenteum* tree survival in a regression model

Predictor	Estimate	Std. Error	Z value	*P*-value
(Intercept)	−1.0624	0.6299	−1.687	0.091
Motherstock _control	−0.2875	0.4308	−0.667	0.505
Silver tree Avenue phosphite treatment	0.2204	0.4094	0.538	0.590
Motherstock phosphite treatment	−0.7476	0.4507	−1.659	0.097
DBH (cm)	0.0588	0.0570	1.032	0.302

Despite providing good protection in a controlled environment, the field injection did not lead to a decrease in mortality. Within 2.5 years, 32% of trees older than 7 years died. This suggests that more than two-thirds of the population might perish within 5 years. Since fires are required for recruitment, the absence of fire and high tree mortality mean the population could disappear within a decade. Since *L. argenteum* is serotinous, seeds remain on the tree, but when a tree dies, seeds are released into mature fynbos vegetation with little chance of successful recruitment, but long-lived soil-stored seed banks might ensure persistence (Heelemann et al. 2013). Despite testing two concentrations of phosphite, both within the range of standard dosages for Proteaceae species (Hardy et al. 2001; Wilkinson et al. 2001; Shearer and Fairman 2007), there was no significant difference in survival between treated and non-treated trees. The widespread observation of *Armillaria* mycelial mats on dead trees, coupled with the isolation and identification of *A. gallica* from one such tree, strongly suggests that Armillaria root rot contributed to mortality. *Armillaria* is a genus

Fig. 6 *Leucadendron argenteum* trees at Kirstenbosch National Botanical Garden with (**a**) collar lesion caused by *Phytophthora cinnamomi* and (**b**, **c**) mycelial mats indicative of *Armillaria* infection

of important root pathogens that affect woody plants worldwide (Wingfield et al. 2010). Notably, Armillaria root rot has been attributed to the decline and death of Proteaceae in the KNBG and *A. mellea* has also been previously isolated from *L. argenteum* at this location (Coetzee et al. 20032018; Wingfield et al. 2010). While only *A. gallica* was recovered during this trial, both species likely occur sympatrically. The dual presence of *P. cinnamomi* and *Armillaria* spp. may explain the unexpected high mortality in phosphite-treated trees.

The results of our glasshouse study on the South African *L. argenteum* aligns with the research on Australian Proteaceae. There was no adverse effect from the phosphite treatment, and there was a significant decrease in disease development in the treated inoculated seedlings. Similarly Wilkinson et al. (2001) demonstrated a significant reduction in *P. cinnamomi* impact across multiple Australian plant species following phosphite application, particularly when administered immediately after infection. However, their study found an effect in both controlled glasshouse experiments and field trials. While the results of our glasshouse trial mirror those of studies conducted on Australian plants from comparable ecosystems, our field trial did not demonstrate phosphite's effectiveness, likely due to the presence of *Armillaria* spp. This finding supports Marçais et al. (2011), who stated that trees in natural environments face multiple pathogens rather than one. The synergy between *Phytophthora* root rot and Armillaria root rot suggests that the former may predispose trees to the latter's infection and subsequent demise. Marcais et al. (2011) observed this phenomenon in a glasshouse trial on pedunculate oak, where combined inoculation of *Phytophthora* spp. and *A. mellea* resulted in greater root damage compared to separate inoculation. Further investigation is warranted to evaluate

synergistic effects between *P. cinnamomi* and *Armillaria* spp. in *L. argenteum* mortality

The benefits of phosphite spraying are well documented, but concerns regarding phosphite spraying in fynbos soils have been raised due to the low soil phosphorus content and presence of phosphorus-sensitive species (Lambers et al. 2013). However, studies from comparable systems in Australia show no significant differences in structural characteristics, species richness, or abundance between phosphite treated and untreated sites (Barrett and Rathbone 2018; Boulle et al. 2023). Therefore, phosphite spraying in fynbos can potentially be applied with minimal negative effects. This study suggests that if high mortality rates of *L. argenteum* are observed in natural or planted populations, phosphite treatment should be considered, as this will significantly enhance survival while being unlikely to have a negative impact.

The findings of the glasshouse trial are significant, demonstrating that phosphite can be used as a management tool for *P. cinnamomi*-infected *L. argenteum* if no *Armillaria* is present. Many other plant species in the natural ecosystems of South Africa are impacted by *P. cinnamomi*, including *Leucospermum, Leucadendron, Sorocephalus,* and *Serruria* (Paap et al. 2023; von Broembsen 1984). *Phytophthora cinnamomi* is likely having an impact on other Proteaceae, and phosphite could be a useful tool in maintaining the health of populations of rare and endangered species in situ, to buy time for the collection of germplasm for ex-situ conservation (Paap et al. 2023). In a study surveying the diversity of *Phytophthora* species in South African botanical gardens, it was found that *P. cinnamomi* was the second most recovered of these species, and is responsible for *L. argenteum* decline (Hulbert et al. 2019). This study demonstrates that phosphite could be a valuable resource for protecting the well-being of other rare and endangered species in habitats infected with *P. cinnamomi*. There are recent studies suggesting that calcium chelate is as effective in controlling *P. cinnamomi* as phosphite, and if used together, these chemicals have a synergistic effect in inhibiting *P. cinnamomi* (Khdiar et al. 2023), but this still must be tested in the CFR. The current study highlights *A. gallica* as a potential threat to Cape flora. Given the historical records of *A. mellea* at the site, and the fact that both species are alien to South Africa, we recommend risk analyses be performed to support their regulatory listing under the NEM:BA A&IS Regulations (South African National Environmental Management: Biodiversity Act [NEM:BA, Act 10 of 2004] Alien and Invasive Species Regulations; Department of Environment Forestry and Fisheries 2020). Further research should also evaluate the efficacy of phosphite application in mitigating Armillaria root rot. For Phytophthora root rot, the results derived from our glasshouse trial are consistent with findings from longer-term trials conducted in Australia (Barrett and Rathbone 2018; Boulle et al. 2023), demonstrating that phosphite offers a viable management tool for *P. cinnamomi* within CFR ecosystems dominated by Proteaceae.

Acknowledgements We thank the South African National Biodiversity Institute (SANBI) and the staff at Kirstenbosch National Botanical Garden for providing access to the study sites, as well as the necessary facilities and resources to conduct both glasshouse and fieldwork experiments. Mesfin Gossa, Reuben Stander and Josiah King are thanked for their assistance in setting up the field trial. This study was funded by the Joan Wrench Scholarship Fund, and the South African Department of Forestry, Fisheries and the Environment (DFFE) noting that this publication does not necessarily represent the views or opinions of DFFE or its employees.

Funding Open access funding provided by Cape Peninsula University of Technology. This study was funded by the Joan Wrench Scholarship Fund, and the South African Department of Forestry, Fisheries and the Environment (DFFE) noting that this publication does not necessarily represent the views or opinions of DFFE or its employees.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval No ethical approval was required.

Competing interests The authors have no competing interests to declare.

References

Akinsanmi OA, Drenth A (2013) Phosphite and metalaxyl rejuvenate macadamia trees in decline caused by *Phytophthora cinnamomi*. Crop Prot 53:29–36. https://doi.org/10.1016/j.cropro.2013.06.007

Barrett S, Rathbone D (2018) Long-term phosphite application maintains species assemblages, richness and structure of plant communities invaded by *Phytophthora cinnamomi*. Austral Ecol 43:360–374. https://doi.org/10.1111/aec.12574

Bose T, Roux J, Burgess TI, Shaw C, Wingfield MJ (2019) Susceptibility of *Eucalyptus grandis* and *Acacia mearnsii* seedlings to five *Phytophthora* species common in South African plantations. For Pathol 49:e12560 https://doi.org/10.1111/efp.12560

Boulle M, Stewart BA, Barrett S (2023) To spray or not to spray: impact of phosphite spraying for *Phytophthora cinnamomi* control on Proteaceae species in southwestern Australia. Conserv Sci and Pract 5:e12903. https://doi.org/10.1111/csp2.12903

Burgess TI, Scott JK, McDougall KL, Stukely MJ, Crane C, Dunstan WA, Brigg F, Andjic V, White D, Rudman T, Arentz F (2017) Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. Glob Chang Biol 23:1661–1674. https://doi.org/10.1111/gcb.13492

Cahill DM, Rookes JE, Wilson BA, Gibson L, McDougall KL (2008) *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. Aust J Bot 56:279–310. https://doi.org/10.1071/BT07159

Coetzee MA, Musasira NY, Roux F, van der Merwe NA, Wingfield MJ (2018) *Armillaria* root rot spreading into a natural woody ecosystem in South Africa. Plant Pathol 67:883–891. https://doi.org/10.1111/ppa.12804

Coetzee MA, Wingfield BD, Roux J, Crous PW, Denman S, Wingfield MJ (2003) Discovery of two northern hemisphere *Armillaria* species on Proteaceae in South Africa. Plant Pathol 52:604–612. https://doi.org/10.1046/j.1365-3059.2003.00879.x

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM (2000) A molecular phylogeny of Phytophthora and related oomycetes. Fungal Genet Biol 30:17–32. https://doi.org/10.1006/fgbi.2000.1202

de Lange JH, Boucher C (1990) Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. S Afr J Bot 56:700–703. https://doi.org/10.1016/S0254-6299(16)31009-2

Department of Environment Forestry and Fisheries 2020 South African National Environmental Management: biodiversity Act [NEM:BA, Act 10 of 2004] Alien and Invasive Species Regulations. Government Gazette, notice 1020 of 2020, 43735: 14–44.

Dunstan WA, Rudman T, Shearer BL, Moore NA, Paap T, Calver MC, Dell B, GESJ H (2010) Containment and spot eradication of a highly destructive, invasive plant pathogen (*Phytophthora cinnamomi*) in natural ecosystems. Biol Invasions 12:913–925. https://doi.org/10.1007/s10530-009-9512-6

Engelbrecht J, Duong TA, Paap T, Hubert JM, Hanneman JJ, van den Berg N (2022) Population genetic analyses of *Phytophthora cinnamomi* reveals three lineages and movement between natural vegetation and avocado orchards in South Africa. Phytopathol 112:1568–1574. https://doi.org/10.1094/PHYTO-10-21-0414-R

Garbelotto M, Schmidt DJ, Harnik TY (2007) Phosphite injections and bark application of phosphite + Pentrabark™ control sudden oak death in coast live oak. Arboric Urban For 33:309. https://doi.org/10.48044/jauf.2007.035

Girden ER (1992) ANOVA: repeated Measures. Sage

Global Invasive Species Database (2024) Species profile: *Phytophthora cinnamomi*. Downloaded from http://www.iucngisd.org/gisd/species.php?sc=143 on 24 May 2024.

Goldblatt P (1997) Floristic diversity in the Cape flora of South Africa. Biodivers Conserv 6:359–377. https://doi.org/10.1023/A:1018360607299

Graham JH (2011) Phosphite for control of *Phytophthora* diseases in citrus: model for management of *Phytophthora* species on forest trees? N Z J For Sci 41:49–56

Hardham AR, Blackman LM (2018) Phytophthora cinnamomi. Mol Plant Pathol 19:260–285. https://doi.org/10.1111/mpp.12568

Hardy GESJ, Barrett S, Shearer BL (2001) The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. Australas Plant Pathol 30:133–139. https://doi.org/10.1071/AP01012

Heelemann S, Daniels F, Rebelo AG, Poschlod P, Reisch C (2013) Conservation genetics of *Leucadendron argenteum* (Silvertree)—A flagship species of the Cape Peninsula. S Afr J Bot 88:361–366. https://doi.org/10.1016/j.sajb.2013.08.014

Hulbert JM, Paap T, Burgess TI, Roets F, Wingfield MJ (2019) Botanical gardens provide valuable baseline *Phytophthora* diversity data. Urban For Urban Green 46:126461. https://doi.org/10.1016/j.ufug.2019.126461

Khdiar MY, Burgess TI, Barber PA, GESJ H (2023) Calcium chelate is as effective as phosphite in controlling *Phytophthora* root rot in glasshouse trials. Plant Pathol 72:112–119. https://doi.org/10.1111/ppa.13642

Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion variance analysis. J Am Stat Assoc 47:583–621. https://doi.org/10.1080/01621459.1952.10483441

Lambers H, Ahmedi I, Berkowitz O, Dunne C, Finnegan PM, GESJ H, Jost R, Laliberté E, Pearse SJ, Teste FP (2013) Phosphorus nutrition of phosphorus-sensitive Australian native plants: threats to plant communities in a global biodiversity hotspot. Conserv Physiol 1:cot010 https://doi.org/10.1093/conphys/cot010

Linde C, Drenth A, Kemp GHJ, Wingfield MJ, Von Broembsen SL (1997) Population structure of *Phytophthora cinnamomi* in South Africa. Phytopathology 87:822–827. https://doi.org/10.1094/PHYTO.1997.87.8.822

Mangiafico SS (2024) Rcompanion: functions to Support Extension Education Program Evaluation. Rutgers Cooperative Extension, New Brunswick, New Jersey Version 2.4.35. https://CRAN.R-project.org/package=rcompanion/

Marçais B, Caël O, Delatour C (2011) Interaction between root rot basidiomycetes and *Phytophthora* species on pedunculate oak. Plant Pathol 60:296–303. https://doi.org/10.1111/j.1365-3059.2010.02378.x

Masikane SL, Novela P, Mohale P, McLeod A (2020) Effect of phosphonate application timing and strategy on phosphite fruit and root residues of avocado. Crop Prot 128:105008. https://doi.org/10.1016/j.cropro.2019.105008

Ogle DH, Doll JC, Wheeler AP, Dinno A (2023) FSA: simple Fisheries Stock Assessment Methods. R package version 0.9.5. https://CRAN.R-project.org/package=FSA/

Olivier D (1951) Progress in the study of the silver-tree disease. Veld and Flora 37:18

Paap T, Nndanduleni M, Wingfield MJ (2023) A critically endangered Proteaceae in the Cape Floristic Region threatened by an invasive pathogen. Bothalia 53:1–7. https://doi.org/10.38201/btha.abc.v53.i1.6

Qongqo A, Nchu F, Geerts S (2022) Relationship of alien species continues in a foreign land: the case of *Phytophthora* and Australian *Banksia* (Proteaceae) in South African Fynbos. Ecol Evol 12:e9100. https://doi.org/10.1002/ece3.9100

R Core Team (2023) R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rands RD (1922) Stripe canker of cinnamon caused by *Phytophthora cinnamomi*. N. Sp. Mededelingen van het Instituut voor Plantenziekten 54

Rebelo AG, Mtshali H, Koopman R, Raimondo D (2020) *Leucadendron argenteum* (L.) R. Br. National Assessment: red List of South African Plants version. http://redlist.sanbi.org/species.php?species=794-8. 24 April 2024

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611. https://doi.org/10.2307/2333709

Shearer BL, Crane CE, Cochrane JA, Dunne CP (2013) Variation in susceptibility of threatened flora to *Phytophthora cinnamomi*. Australas Plant Pathol 42:491–502. https://doi.org/10.1007/s13313-013-0215-1

Shearer BL, Fairman RG (2007) A stem injection of phosphite protects Banksia species and *Eucalyptus marginata* from *Phytophthora*

cinnamomi for at least four years. Australas Plant Pathol 36:78–86. https://doi.org/10.1071/AP06085

Summerell B, Liew E (2020) Phytophthora root rot: its impact in botanic gardens and on threatened species conservation. Sibbaldia 18:89–104. https://doi.org/10.24823/Sibbaldia.2020.290

Tchotet Tchoumi JM, Coetzee MPA, Vivas M, Rajchenberg M, Roux J (2017) Wood-rotting basidiomycetes associated with declining native trees in timber-harvesting compartments of the Garden Route National Park of South Africa. Austral Ecol 42:947–963. https://doi.org/10.1111/aec.12524

Tkaczyk M, Kubiak KA, Sawicki J, Nowakowska JA, Oszako T (2016) The use of phosphates in forestry. Forest Research Papers 77:76–81. https://doi.org/10.3390/f15050829

Tobin PC (2015) Ecological consequences of pathogen and insect invasions. Curr For Rep 1: 25–32. https://doi.org/10.1007/s40725-015-0008-6

van Wyk PS (1973) Root and crown rot of silver trees. J S Afr Bot 39:255–260

von Broembsen S (1984) Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. Phytophylactica 16:221–226

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.) PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, pp 315–322

Wickham H (2016) ggplot2: elegant Graphics for Data Analysis. Springer-Verlag, New York. https://ggplot2.tidyverse.org/

Wilkinson CJ, Holmes JM, Tynan KM, Colquhoun IJ, McComb JA, Hardy GSJ, Dell B (2001) Ability of phosphite applied in a glasshouse trial to control *Phytophthora cinnamomi* in five plant species native to Western Australia. Australas Plant Pathol 30:343–351. https://doi.org/10.1071/AP01055

Wingfield MJ, Coetzee MP, Crous PW, Six D, Wingfield BD (2010) Fungal phoenix rising from the ashes? IMA Fungus 1:149–153. https://doi.org/10.5598/imafungus.2010.01.02.06