Fungicide Sensitivity of *Trichoderma atroviride* and the Application of this Biocontrol Fungus to Protect Grapevine Sucker Wounds

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It is known that *Trichoderma* spp. can protect winter pruning wounds effectively against infection by trunk disease pathogens. Another port of entry for trunk disease pathogens is spring sucker wounds. *Trichoderma* spp. hold the potential to be applied to grapevine sucker wounds to prevent infection by trunk disease pathogens. During spring, fungicides are regularly applied to control grapevine foliar and fruit diseases. The effect of these fungicides on *T. atroviride* is unknown. The efficacy of a *T. atroviride*-based commercial product, Eco-77®, was tested on sucker wounds in a vineyard against the trunk pathogens *Phaeomoniella chlamydospora* and *Diaporthe ampelina*. Sucker wounds were made on one-year-old wood of Cabernet Sauvignon vines. The wounds were spray-treated with Eco-77® immediately after suckering, and 24 hours later the sucker wounds were inoculated with spore suspensions of either *P. chlamydospora* or *D. ampelina*. After five months, isolations were made from the sucker wounds. *Trichoderma atroviride* reduced the incidence of *P. chlamydospora* by 66.65%. Even though the incidence of *D. ampelina* was reduced by 15.37%, it was not significantly different from the control treatment. *In vitro* sensitivity studies were utilised to investigate the effect of fungicides applied during spring against other diseases (downy and powdery mildew, Botrytis rot and Phomopsis cane and leaf spot) on *Trichoderma* spp. The inhibition of mycelial growth and conidial germination of *T. atroviride* (UST1 and Eco-77®) were screened for 16 fungicides. Potato dextrose agar and broth was amended to obtain 0, 0.25, 0.5, 1 and 2 times the recommended dosages of the respective fungicides. *Trichoderma* spp. isolates were the least sensitive to the systemic fungicides boscalid, metrafenone and trifloxystrobin, as well as to the contact fungicides quinoxyfen and meptyldinocap for mycelial inhibition. These fungicides gave less than 50% mycelial inhibition at all the tested dosages. For the conidial germination assay, boscalid, penconazole and trifloxystrobin (systemic fungicides) gave less than 50% conidial germination inhibition of *Trichoderma* spp. Spiroxamine and pyrimethanil gave the highest mean percentage inhibition for both mycelial inhibition and conidial germination. The findings of this study show that *T. atroviride* can potentially be used to protect sucker wounds against *P. chlamydospora*. Furthermore, fungicides applied to grapevines during springtime can influence the conidial germination and mycelial growth of *T. atroviride* (UST1 and Eco-77®) to varying degrees. When applying *Trichoderma* spp. in spring, care needs to be taken in the timing of application of fungicides so as not to inhibit the growth of the biocontrol fungus.

INTRODUCTION

The pruning of grapevines is an annual practice that is performed during dormancy to ensure a balance between productivity and vegetative growth. Since pruning results in the exposure of xylem vessels, abundant rainfall and increased inoculum availability during dormancy lead to infections by grapevine trunk disease pathogens (Moller & Kasimatis, 1978; Serra *et al*., 2008; Van Niekerk *et al*., 2011; Gramaje *et al*., 2018). Winter pruning wounds are considered to be the main infection portals for xylem-inhabiting trunk pathogens, which cause dieback and the decline of grapevines. Grapevine trunk diseases are caused by a variety of fungal pathogens, including species of *Diatrypaceae* (Eutypa dieback) (Moyo *et al*., 2018), *Phaeomoniella chlamydospora* and *Phaeacremonium* species (Petri disease and esca) (Cloete *et al*., 2015), wood-rotting fungi from the Hymenochaetales (esca) species of the *Botryosphaeriaceae* (Botryosphaeria dieback and canker) (Van Niekerk *et al*., 2006) and *Diaporthe* spp. (Phomopsis dieback) (Mugnai

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et al., 1999; Mostert *et al*., 2001; Fischer, 2002; Urbez-Torres *et al*., 2006; Trouillas *et al*., 2011; Lesuthu *et al*., 2019). Apart from infecting winter pruning wounds, it has been shown that *E. lata* (Lecomte & Bailey, 2011) and *Diplodia seriata* (Epstein *et al*., 2008) can infect sucker wounds made during spring.

To date, most research has focused on the role of winter pruning wounds in trunk disease epidemiology, which means that the significance of sucker wounds remains uncertain. Lecomte and Bailey (2011) concluded that sucker wounds may provide a risk of grapevine infection by *E. lata*, although this risk is less than in the case of winter pruning wounds. Epstein *et al*. (2008) also found sucker wounds to be naturally susceptible to *D. seriata* on vines in the field. Furthermore, Makatini *et al*. (2023) isolated *Diaporthe ampelina*, *Diplodia seriata*, *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, *Eutypella microtheca*, *Cryptovalsa ampelina* and *Neofusicoccum australe* from sucker wounds. Through artificial inoculation, these authors (Makatini *et al*., 2023) demonstrated that *E. lata*, *N. parvum*, *Pm. minimum*, *P. chlamydospora* and *D. ampelina* can infect green shoot wounds. Sosnowski and Ayres (2022) demonstrated that spring shoot-thinning wounds, made by tearing off green shoots, can become infected when inoculated with *E. lata* and *D. seriata* spores. Winter pruning wounds are most susceptible for the first two weeks after pruning, but can remain susceptible for up to 16 weeks, with the susceptibility declining as the wounds age (Petzoldt *et al*., 1981; Munkvold & Marois, 1995; Eskalen *et al*., 2007; Van Niekerk *et al*., 2011; Elena & Luque, 2016; Díaz & Latorre, 2022). The protection of winter pruning wounds is especially important for the first two-week period after pruning, since sucker wounds are susceptible to trunk disease pathogens. It is reasonable to suggest that they also need to be protected from trunk disease infections.

Pruning wound protection, including fungicides, paints and pastes, form the basis of chemical control for wound protection. Fungicides protect wounds for approximately 14 days, and not for the full period of pruning wound susceptibility (Munkvold & Marois, 1993b; Weber *et al*., 2007). In comparison with fungicides, biocontrol applications provide long-term pruning wound protection. *Trichoderma* spp. have received the most attention as biocontrol agents (BCA) because this genus has shown good potential in protecting wounds. On grapevines, *Trichoderma* spp. have been shown to penetrate (Harvey & Hunt, 2006; McLean *et al*., 2009) and protect wounds against *E. lata* (Carter 1983; John *et al*., 2005; Halleen *et al*., 2010; Mutawila *et al*., 2011b; Blundell & Eskalen, 2022) and other trunk disease fungi for at least eight to 12 months (Halleen *et al*., 2010; Mutawila *et al*., 2011a). The mechanisms of control used by *Trichoderma* spp. include competitive exclusion, mycoparasitism and antibiosis, which occur when *Trichoderma* spp. compete with other pathogens for space, nutrients and water (Benítez *et al*., 2004; John *et al*., 2005; Kotze *et al*., 2011).

There are some challenges that limit the use of *Trichoderma* spp. as BCAs for wound protection. *Trichoderma* spp. require time to colonise wounds and therefore there is a window period of susceptibility prior to establishment (John *et al*., 2005; Mutawila *et al*., 2016). The timing of application with regard to the vine's physiological status is also crucial, because propagules of the fungus may be washed off by xylem sap during vine 'bleeding', which consequently will lead to poor establishment (Mutawila *et al*., 2016). *Trichoderma* spp. may also be incompatible with fungicides, making it difficult to incorporate them into integrated pest management strategies. For grapevines, many chemical sprays are applied against diseases such as powdery and downy mildew, botrytis rot and Phomopsis cane and leaf spot during suckering in spring and early summer. However, the sensitivity of *Trichoderma* spp. towards these fungicides is unknown.

Therefore, the objectives of the current study were to i) evaluate *Trichoderma atroviride* against the trunk pathogens *P. chlamydospora* and *D. ampelina* on sucker wounds in the field and ii) determine the sensitivity of two *T. atroviride* isolates *in vitro* against 16 fungicides that are used to control powdery mildew, downy mildew, botrytis rot and Phomopsis cane and leaf spot.

MATERIALS AND METHODS

Application of *Trichoderma atroviride* to sucker wounds in the field

A *Trichoderma atroviride*-based pruning wound product, Eco-77®, was kindly provided by Andermatt Plant Health Products (Andermatt PHP, PTY Ltd., Nottingham Road, South Africa). Eco-77® was applied at the recommended rate of 0.5g/L. *Phaeomoniella chlamydospora* and *D. ampelina* are maintained in the culture collection of the Department of Plant Pathology at Stellenbosch University, as STE-U 6384 and STE-U 7768, respectively. *Phaemoniella chlamydospora* conidial suspension was prepared from a two-week-old fungal culture grown on potato dextrose agar (PDA, Biolab, Wadeville, South Africa). A conidial suspension of *D. ampelina* was made by suspending conidial droplets from pycnidia that formed on four-week-old fungal cultures on PDA with sterilised pine needles.

A 12-year-old Cabernet Sauvignon vineyard trained to bilateral cordons on a horizontally divided trellis situated in Stellenbosch was used for field inoculations. During July 2012 (winter), the vines were spur pruned to five buds. In October 2012 (spring), sucker wounds were created by removing the second shoot (50 mm to 70mm in length) below the pruning wound of the one-year-old canes. Wounds were then spray-treated with Eco-77® by means of a hand-held trigger spray canister. After two days, sucker wounds were inoculated with 1 000 spores of *P. chlamydospora* and *D. ampelina* conidial suspensions. Treatments included Eco-77®, *D. ampelina*, *P. chlamydospora*, plus a combination (Eco-77® + pathogen), with sterile dH_2O as a control. The trial was laid out in a completely randomised block design with three blocks that consisted of ten vines each. Each vine received all six treatments – one treatment per spur. Five months later, sucker wounds were excised (leaving approximately 2 cm above and below the sucker wound) and taken to the laboratory for fungal re-isolations and identification.

Fungal isolations from sucker wounds were surface-disinfected by dipping into 70% ethanol for 30 seconds, then one minute in 3.5% sodium hypochlorite solution, and again

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in 70% ethanol for 30 seconds. Wounds were aseptically dissected longitudinally, and wood fragments were taken from the wound scar interphase and 5 mm away from the first isolation position. In total, eight wood pieces were excised – four wood fragments (5 mm x 1 mm) from each isolation position on each half. The wood fragments were plated onto 90 mm Petri dishes containing PDA amended with chloromycetin (250 mg/L). Petri dishes were incubated at approximately 25°C and monitored for four weeks. Fungal cultures were identified based on cultural and morphological characters as *P. chlamydospora* (Crous & Gams, 2000), *D. ampelina* (Mostert *et al*., 2001; Van Niekerk *et al*., 2005) and *Trichoderma* (Gams & Bisset, 1998).

The incidence of *T. atroviride*, *P. chlamydospora* and *D. ampelina* was calculated by the presence or absence of these fungi per sucker wound for each treatment. The data was subjected to analysis of variance (ANOVA) and the Student's t-test for the least significant differences (LSD) at the 5% level of significance ($P < 0.05$). The differences in the pathogen incidences of individual and combined treatments were sought by ANOVA. Analysis was performed using SAS version 9.2 statistical software (SAS Institute Inc, SAS Campus Drive, Cary, North Carolina, USA).

***In vitro* fungicide sensitivity testing of *T. atroviride* isolates**

In vitro assays were performed using two *Trichoderma* spp. Isolates, UST1 (*T. atroviride*) and Eco-77® (*T. atroviride*). Both isolates were used for mycelial inhibition and conidial germination tests. Isolate UST1 is maintained in the culture collection of the Department of Plant Pathology Stellenbosch University, as STE-U 6514. Sixteen commercial fungicides were screened, including contact and systemic products that are used for the control of powdery (*Uncinula necator*) and downy mildew (*Plasmopara viticola*), botrytis rot (*Botrytis cinerea*) and Phomopsis cane and leaf spot (*D. ampelina*) (Table 1).

Inhibition of mycelial growth

All 16 fungicides were screened *in vitro* for the mycelial inhibition of Eco-77® and UST1. Stock solutions were made by suspending fungicides directly into 1 000 ml of sterile dH_2O. Fungicide solutions were then pipetted in

TABLE 1
Fungicides used against *Botrytis cinerea*, *Diaporthe ampelina*, *Plasmopara viticola* and *Erysiphe necator*, selected for screening *in vitro* compatibility with *Trichoderma atroviride*.

No.	Active	Chemical group according to the FRAC code list (2022)	Mode of action	Recommended against	Concentration of active	Dosage ppm = mg/L
1	spiroxamine	5 - SBI class II	Systemic	*E. necator*	500 g/L	300
2	boscalid	7 - carboxamide	Systemic	*E. necator*	500 g/kg	400
3	penconazole	3 - DMI	Systemic	*E. necator*	100 g/L	22.5
4	flusilazole	3 - DMI	Systemic (protective and curative)	*E. necator*	100 g/L	35
5	metrafenone	U8 - benzophenone	Locally systemic and Contact	*E. necator*	500 g/L	125
6	trifloxystrobin	11 - QOI	Systemic and contact	*D. ampelina*	500 g/kg	200
				E. necator		
7	fenarimol	3 - DMI	Systemic	*E. necator*	11.60%	24
8	quinoxyfen	13 - quinolene	Contact	*E. necator*	250 g/L	62.5
9	meptyldinocap	29 - dinitrophenol	Contact	*E. necator*	35.71%	140
10	mancozeb	M3 - dithiocarbamate	Contact	*D. ampelina*	750 g/kg	150
				P. viticola		
11	copper hydroxide	M1 - inorganic	Contact	*P. viticola*	538 g/L	807
12	folpet	M4 - phthalamides	Contact	*D. ampelina*	80% w/w	1 000
13	propineb	M3 - dithiocarbamate	Contact	*P. viticola*	70% a.i./kg	2 100
14	pyrimethanil	9 - AP	Contact	*B. cinerea*	400 g/L	800
15	metiram	M3 - dithiocarbamate	Contact	*P. viticola*	700 g/kg	1 400
				D. ampelina		
16	diathanon	M9 - quinones	Contact	*P. viticola*	700 g/kg	525
				D. ampelina		

Notes: SBI = sterole biosynthesis inhibitors; DMI = demythylation inhibitors; QOI = quinone outside inhibitors; AP = anilino-pyrimidines.

appropriate quantities to bottles that contained molten PDA at approximately 50°C to achieve 0.25, 0.5, one and two times the recommended dosages (Table 1). Potato dextrose agar without fungicide was used as a control treatment. Plates were inoculated within 24 hours with mycelium plugs of 5 mm diameter obtained from the margins of actively growing seven-day-old cultures of Eco-77® and UST1. Each fungicide concentration, as well as the controls, were replicated three times and the trial was repeated. Petri dishes were incubated at 25°C for three days. At 24 hours, the diameter of each colony was measured twice at perpendicular angles. For each isolate × fungicide × concentration combination, the percentage inhibition was calculated in relation to the respective control treatment. The percentage inhibition was calculated as follows: 100 × [(colony diameter of control) - (colony diameter on fungicide-amended plate) / (colony diameter of control)]. The fungicides were regarded as being compatible with *Trichoderma* isolates if they gave less than 50% mean percentage inhibition at all the tested dosages.

Inhibition of conidial germination

The inhibition of conidial germination of Eco-77® and UST1 were tested against all 16 fungicides at the recommended dosages (Table 1). Spore suspensions were prepared by flooding seven-day-old PDA cultures of Eco-77® and UST1 with 5 ml sterile water. The concentrations were adjusted to 1 x 10^5 spores per ml in potato dextrose broth (PDB, Biolab, Wadeville, South Africa) using a haeomocytometer. Aliquots (0.5 ml) of spore suspension and fungicide (0.5 ml) were added to 1.5 ml Eppendorf tubes. Sterile dH_2O was used as a negative control treatment. Each spore-fungicide mix was replicated three times and the trial was repeated. Tubes were placed in a shaker incubator (100 rpm) at 25°C. After 24 h, three droplets were taken separately from each tube and viewed under a light microscope (× 400, Zeiss, West Germany). Spores were considered to have germinated if the germ tube length equalled the spore diameter. The percentage inhibition was recorded for 50 spores per sample, and the mean percentage inhibition relative to the control was calculated per fungicide. The percentage inhibition was calculated as follows: 100 × [(number of germinated spores in control tubes) - (number of germinated spores in fungicide-amended tube) / (number of germinated spores in control tubes)].

Data analysis

For the mycelial inhibition, the percentage inhibition of both experiments was pooled. The data was subjected to analysis of variance (ANOVA) and the Student's t-test for the least significant differences (LSD) at the 5% level of significance ($P < 0.05$). Significant differences in conidial inhibition between the fungicides were determined using a one-way ANOVA. Analyses were performed using SAS version 9.2 statistical software (SAS Institute Inc, SAS campus Drive, Cary, North Carolina, USA).

RESULTS

Application of *Trichoderma atroviride* to sucker wounds in the field

The analysis of variance revealed a significant ($P = 0.03$) difference between *P. chlamydospora* treatments. The mean incidence of *Phaeomoniella chlamydospora* decreased by 66.65% when it was inoculated on Eco-77® treated sucker wounds (Table 2). There were no significant differences between *D. ampelina* treatments ($P = 0.07$) – the mean incidence of *D. ampelina* (Table 2) decreased by 15.37% when it was inoculated on wounds treated with Eco-77®. Re-isolation of *T. atroviride* from the control treated sucker wounds was 25%.

***In vitro* fungicide sensitivity testing of *T. atroviride* isolates**

Mycelial inhibition

Analysis of variance revealed a significant isolate × concentration × fungicide interaction ($P < 0.0001$) for mycelial inhibition for both *Trichoderma* isolates at 24 and 48 hours. All 16 fungicides inhibited the growth of *Trichoderma* to some degree at all the tested concentrations after 24 and 48 hours. The mean percentage inhibition generally increased with an increase in concentration, whilst the sensitivity of isolates to most fungicides generally decreased with an increase in time. At 0.25 × (Fig. 1), *Trichoderma* isolates were only sensitive to spiroxamine and penconazole (systemic), and to all contact fungicides except for quinoxyfen, meptyldinocap and metiram (UST1) after 24 hours. At 0.5 × (Fig. 1), *Trichoderma* isolates were only sensitive to spiroxamine, flusilazole and fenarimol (Eco-77® only) (systemic), and to all contact fungicides except quinoxyfen and meptyldinocap after 24 hours. At the recommended dosage (Fig. 1), *Trichoderma* isolates were sensitive to all systemic fungicides except boscalid, metrafenone and trifloxystrobin, and to all contacts except quinoxyfen and meptyldinocap after 24 hours. At 2 ×

TABLE 2

Mean incidence of *Phaeomoniella chlamydospora* and *Diaporthe viticola* re-isolated from sucker wounds of Cabernet Sauvignon vines five months after sucker wounds were inoculated with *Trichoderma atroviride* (Eco-77®) and two days later with *Phaeomoniella chlamydospora* or *Diaporthe ampelina*.

Treatment	Mean percentage incidence (%)	
	Phaeomoniella chlamydospora*	***Diaporthe ampelina**#*
Pathogen only	20.00[a]	43.33[a]
T. atroviride + pathogen	6.67[b]	36.67[a]

Means followed by the same letter are not significantly different ($P > 0.05$; *LSD = 13.32; #LSD = 39.40)

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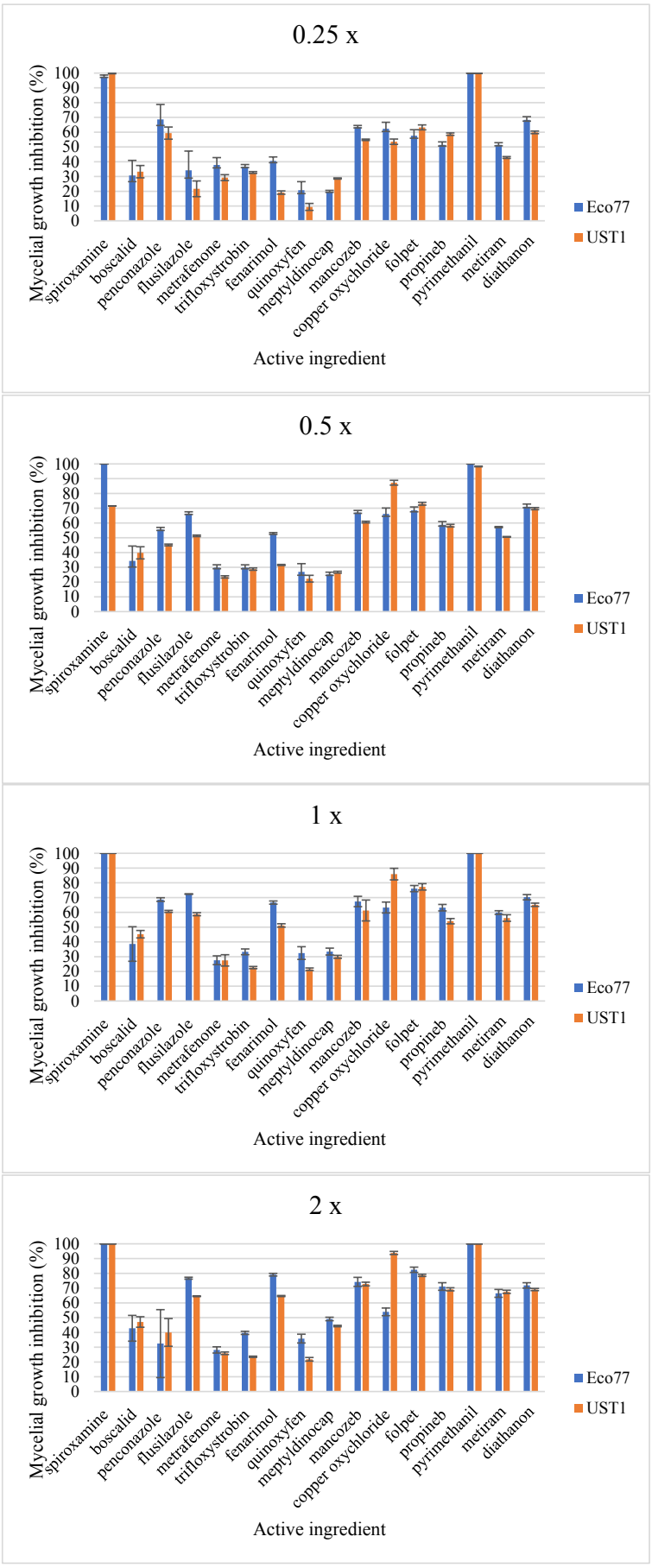

FIGURE 1
The percentage mycelial growth inhibition of Trichoderma species isolates caused by fungicides at 0.25, 0.5, one (recommended dosage) and two times the recommended dosage after incubation for 24 hours at 25°C.

(Fig. 1), isolates were sensitive to spiroxamine, flusilazole and fenarimol (systemic), and to all contact fungicides except quinoxyfen and meptyldinocap. Similar trends were observed after 48 hours (data not shown) for all the concentrations. *Trichoderma atroviride* isolates were least sensitive to the systemic fungicides boscalid, metrafenone and trifloxystrobin, as well as to the contact fungicides quinoxyfen and meptyldinocap.

Inhibition of conidial germination

Analysis of variance revealed significant isolate × fungicide ($P < 0.0001$), fungicide ($P < 0.0001$) as well as isolate ($P < 0.0001$) interactions. The following fungicides – boscalid (for Eco-77), penconazole and trifloxystrobin (systemic) – inhibited less than 50% of conidial germination (Fig. 2). No conidia germinated in the presence of spiroxamine (systemic) and mancozeb, propineb, metiram and diathon (contact). The fungicides with the highest inhibition were therefore all contact fungicides (mancozeb, propineb, metiram, pyrimethanil and diathanon), except for spiroxamine, which is systemic.

DISCUSSION

This study has demonstrated the potential use of *Trichoderma atroviride* for sucker wound protection against the trunk pathogens *P. chlamydospora* and *D. ampelina*. The application of *Trichoderma* products to sucker wounds has not been reported previously. *Trichoderma atroviride* colonised sucker wounds and could be re-isolated after five months. The BCA significantly decreased the incidence of *P. chlamydospora* in sucker wounds when it was applied as a wound treatment prior to pathogen inoculation.

Trichoderma atroviride reduced the incidence of *P. chlamydospora* by 66.65% in sucker wounds that were treated with Eco-77®. Various field trials have shown that *Trichoderma* spp. can inhibit the infection of winter pruning wounds by the trunk disease fungi *E. lata* (Carter & Price, 1974; Carter, 1983; Munkvold & Marois, 1993a; John *et al*., 2005; Halleen *et al*., 2010; Mutawila *et al*., 2011b; Mutawila *et al*., 2016) and *P. chlamydospora* (Mutawila *et al*., 2011b). In Halleen *et al*. (2010), *Trichoderma* treatments also reduced the incidence of natural infections by *Botryosphaeriaceae* spp., *P. chlamydospora*, *Phaeoacremonium* spp. and *Diaporthe* spp. and, moreover, Eco-77® significantly reduced the total pathogen count in pruning wounds. In the present study, although *T. atroviride* also reduced the incidence of *D. ampelina* by 15.37%, this reduction was not statistically significant. A longer lag period between the application of *Trichoderma* and *D. ampelina* could have improved the efficacy of the *Trichoderma*. When *D. ampelina* was applied seven days after the application of Eco-77® to winter pruning wounds, the incidence of *D. ampelina* was reduced from 34.82% to 18.48% (Kotze *et al*., 2011). John *et al*. (2005) also found that delaying the inoculation of *E. lata* by 14 days after treating wounds with *T. atroviride* and *Fusarium lateritium* increased the efficacy of BCAs and significantly reduced the re-isolation incidence of *E. lata*. It therefore was suggested that BCAs need an establishment period for colonising wound surfaces. The fact that *Trichoderma* did not strongly inhibit the infection of *D. ampelina* in sucker wounds as well as in one-year-old grapevines might indicate that this pathogen was more competitive in infecting active xylem tissue than *Trichoderma*. *Diaporthe ampelina* is well known to infect green shoots and cause Phomopsis cane and leaf spot. *In vitro* studies by Kotze *et al*. (2011) showed that, of the 10 *Trichoderma* spp. isolates tested, only one was observed to inhibit the growth of *D. ampelina* at the microscopic level. It would then seem that *Trichoderma* spp. have a limited effect on *D. ampelina*; however, this would have to be ascertained by testing multiple isolates of *D. ampelina in vitro* and in the field.

When *Trichoderma atroviride* was applied to sucker wounds as an individual treatment, the mean incidence of sucker wounds was 25%. Low incidences could be ascribed to the presence of vascular 'bleeding' that most likely washed off conidia from the wound surface, as has been found with

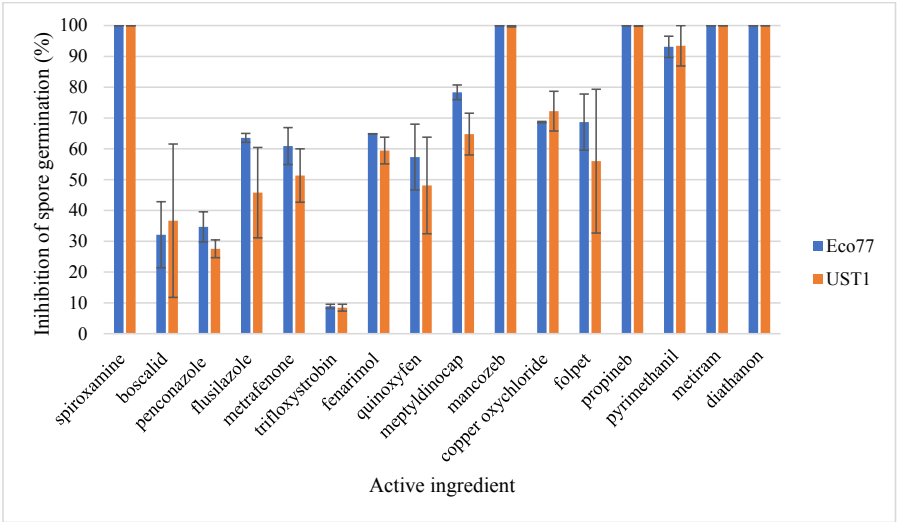

FIGURE 2

The percentage inhibition of spore germination of Trichoderma species isolates caused by fungicides at the recommended dosage after being incubated for 24 hours in a shaking incubator at 25°C.

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pruning wounds (Munkvold & Marois, 1995; John *et al*., 2005; Halleen *et al*., 2010; Mutawila *et al*., 2016). Harvey and Hunt (2006) also obtained poor re-isolation incidences of 25% and 50% when *T. atroviride* was applied between 15 and 30 minutes after pruning, respectively. In the present study, sap flow was observed on the day of and the day following suckering. This may have led to poor establishment of *T. atroviride*, which was applied immediately after suckering. Since grapevines are physiologically active during spring, stronger plant defence could have reduced colonisation by *T. atroviride*, as suggested by Lecomte and Bailey (2011), for the lower infection levels found from sucker wounds versus winter pruning wounds. Another reason for the low sucker wound colonisation by *Trichoderma* may be due to cultivar differences. The inoculation of Eco-77® onto winter pruning wounds of Cabernet Sauvignon gave rise to a 27.5% incidence of *T. atroviride*, lower than for the other cultivars tested, when isolated eight months after inoculation in the field (Mutawila *et al*., 2011a).

Trichoderma spp. have been isolated from winter pruning wounds eight to 12 months after applying the BCA (Halleen *et al*., 2010; Mutawila *et al*., 2011a). The current study demonstrated that *Trichoderma* may provide long-term protection of sucker wounds because it was re-isolated after five months. Fungicides can protect wounds for approximately two weeks (Creasar & Wicks, 2002; Sosnowski *et al*., 2004, 2008). For both winter pruning and sucker wounds, the application of *Trichoderma* spp. holds the advantage of providing protection over the period of wound susceptibility.

In vitro mycelial inhibition and conidial germination tests revealed the sensitivity of *T. atroviride* (UST1) and *T. atroviride* (Eco-77®) to fungicides that are applied during spring. Isolate UST1 appeared to be generally less sensitive to fungicides than Eco-77® for mycelial inhibition and conidial germination. Fungicides that inhibited the mycelial growth and conidial germination of *Trichoderma* isolates by less than 50% can possibly be applied shortly after each other in the field. The systemic fungicides boscalid, metrafenone and trifloxystrobin as well as contacts quinoxyfen and meptyldinocap displayed compatibility with *Trichoderma* isolates inhibiting less than 50% of the mycelial growth at all the tested concentrations. In contrast, spiroxamine and pyrimethanil inhibited *Trichoderma* spp., and the percentages were frequently more than 90%. For the conidial germination, boscalid, penconazole and trifloxystrobin (systemic) inhibited less than 50% of conidial germination.

All of the fungicides inhibited both *T. atroviride* isolates (UST1 and Eco-77®) to a lesser or greater degree. There appeared to be an initial decline in the growth rate of *Trichoderma* mycelium in fungicide treatments after 24 hours; however, a recovery was observed after 48 hours. The overall increase in mycelial inhibition that was observed with an increase in fungicide concentrations in the current study was also observed by Sarkar *et al*. (2010) and Tapwal *et al*. (2012).

Only a few of the fungicides used in the current study have been tested against *Trichoderma* spp.. Various studies have shown that *Trichoderma* spp. are highly sensitive to mancozeb. McLean *et al*. (2001) reported a 100% inhibition of *T. atroviride* by mancozeb, similar to the results of this study. These results are also in agreement with Gupta *et al*. (1995), who reported an inhibition of *T. viride* isolates by mancozeb *in vitro*. Bagwan (2010), however, reported that copper oxychloride and mancozeb were safe to use with *T. atroviride* and *T. viride*. Figueras-Roca *et al*. (1996) reported that fenarimol had low inhibition towards five *Trichoderma* spp. (*T. hamatum*, *T. atroviride*, *T. koningii*, *T. reesei* and *T. saturnisporum*). The conidial germination results of this study also suggest that fenarimol had a low inhibition of *Trichoderma* spp.

It is evident from the results that *Trichoderma* isolates were more sensitive to multi-site contact than systemic fungicides. This was attributed to the fact that multi-site fungicides work against multiple metabolic sites (McGrath, 2004), whereas single-site fungicides only interfere with one of the numerous metabolic pathways, enzymes or proteins that are required by the fungus. Two of the fungicides for powdery mildew with a single-site mode of action, namely boscalid and trifloxystrobin (also registered for Phomopsis cane and leaf spot), were less inhibitive towards *Trichoderma*. These fungicides have the potential to be used at the same time when applying *Trichoderma* spp. in spring to protect sucker wounds.

CONCLUSIONS

Due to increased grapevine trunk diseases worldwide, and the need to find more sustainable means of crop protection, pruning wound protection with BCAs is increasingly becoming the focus of trunk disease research. Since *Trichoderma* spp. are the only registered agents for wound protection in South Africa, the current study investigated their efficacy on sucker wounds. The results of this study demonstrated for the first time in grapevines the ability of *Trichoderma* to protect sucker wounds infected by *P. chlamydospora*, which is an important trunk disease pathogen in South African vineyards. Furthermore, the inhibition of mycelial growth and conidial germination assays of *Trichoderma* spp. showed that the fungicides boscalid and trifloxystrobin could be applied in a close time range with the application of *Trichoderma* spp. to sucker wounds during spring. Further field trials need to be conducted to test the application of *Trichoderma* spp. in the prevention of infection by a wider range of trunk disease pathogens.

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