Effect of *Trichoderma* treatments on the occurrence of decline pathogens in the roots and rootstocks of nursery grapevines

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Summary. The growth-stimulating attributes of *Trichoderma* treatments (dips, soil amendments and drenches with *Trichoderma* products containing propagules of selected strains of *Trichoderma harzianum*) in grapevine nurseries, and their effect on the occurrence of fungi in roots and rootstocks of nursery grapevines, in particular fungi causing Petri disease (*Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.) and black foot rot (*Cylindrocarpon* spp.), were compared with quintozene/procymidone treated (standard) vines. Early shoot growth of *Trichoderma* treated vines was visibly better than that of the control vines. Eight months after planting, at uprooting, percentage take and shoot mass of *Trichoderma* and standard treated vines were similar, but total root mass was significantly higher for *Trichoderma* treated vines. Low percentages of *Cylindrocarpon* spp. were isolated from the rootstocks of treated and untreated vines, while less Petri disease fungi were isolated from rootstocks of *Trichoderma* treated vines. Markedly fewer fungi were also isolated from the roots of *Trichoderma* treated vines. Incidences of Petri disease fungi in roots of *Trichoderma* and standard treated vines were similar, but fewer *Cylindrocarpon* spp. were isolated from *Trichoderma* treated vines. These results indicate the potential of *Trichoderma* treatments in grapevine nurseries for the production of stronger vines with lower *Phaeomoniella*/*Phaeoacremonium* and *Cylindrocarpon* infection levels.

Key words: *Phaeomoniella*, *Phaeoacremonium*, *Cylindrocarpon*, Petri disease, black foot.

Introduction

Wine, table and raisin grape cultivars in South Africa are grafted onto rootstock cultivars to overcome certain biological (phylloxera, nematodes, fungi and bacteria), physical (poor drainage, shallow soils, etc.) and/or chemical (high or low pH, high salinity, etc.) problems in the soil. Propagation material is taken from certified rootstock and scion mother blocks. Grafting is done during late winter to early spring, and graftlings are planted in grapevine nurseries where they grow for one season. The vines are uprooted during winter and sold to wine, table or raisin grape producers. Standard practices to prevent decay by saprophytic fungi involve the treatment of propagation material with contact fungicides (like PCNB, captan or dicarboximides) or sterilising agents (quaternary ammonium compounds) as drenches or dips at various stages before and after grafting.

The origin of two decline diseases of young grapevine in South Africa has been traced to nurseries. The causal fungi of Petri disease[(1)] (also

[(1)] At the general Assembly of the 2nd ICGTD meeting held in Lisbon 2001 it was unanimously decided that young grapevine decline, ‘black goo’, Petri vine decline will henceforth be called Petri disease.

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known as black goo decline), *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. (Scheck *et al.*, 1998; Rego *et al.*, 2000; Groenewald *et al.*, 2001), were found to be present in grapevine rootstock propagation material, whereas the causal fungi of black foot rot, *Cylindrocarpon* spp. (Grasso and Magnano di San Lio, 1975; Sweetingham, 1983; Scheck *et al.*, 1998; Rego *et al.*, 2000), infected the roots and rootstocks of grafted vines from nursery soils (Halleen and Crous, 2001).

Trichoderma soil amendments have been heralded as beneficial to plant growth (Chang *et al.*, 1986; Windham *et al.*, 1986; Chet, 1987; Harman, 2000), antagonistic to soil pathogens (Chet, 1987; Smith *et al.*, 1990; Nemec *et al.*, 1996; Harman, 2000), and possible effectors of induced resistance (Calderon *et al.*, 1993; De Meyer *et al.*, 1998; Harman, 2000). Grapevines with better developed roots and shoots will also be better equipped to withstand stress situations, and consequently should be more resistant to stress-related diseases, in particular Petri disease (Ferreira *et al.*, 1999). The use of *Trichoderma* suspensions and soil amendments is advocated in some grapevine nurseries in Australia and New Zealand (Messina, 1999).

The purpose of this paper is to report on the growth-stimulating attributes of *Trichoderma* treatments (dips, soil amendments and drenches) in a South African nursery, and their effect on the occurrence of fungi, in particular Petri disease and black foot rot causing fungi, in the roots and rootstock of nursery grapevines.

Materials and methods

The trial was performed in a commercial nursery at Wellington in the Western Cape of South Africa. Experimental layout was a completely randomised design with two treatments and 4 repetitions. Rootstock material ('Ramsey') was dipped (5 s) in a *Trichoderma* suspension (Trichoflow-T™, selected strains of *T. harzianum*, Agrimm Technologies Ltd., Christchurch, New Zealand) directly prior to grafting. 'Sauvignon blanc' was hand-grafted to the treated rootstocks, and the graftlings were dipped in *Trichoderma* again. The standard treatments (control) consisted of dips in a mixture of quintozene (PCNB 750 WP, Plaaskem, Johannesburg, South Africa; 500 g/100 l) and procymidone (Sumisclex 250 SC, Sanachem, Johannesburg, South Africa; 200 ml/100 l) at the stages mentioned. *Trichoderma* treated and control graftlings were stacked in separate callusing boxes, covered with sawdust and stored in a callus room (8 to 15°C) for 32 days. Prior to planting, *Trichoderma* pellets (Trichopel-R™, selected strains of *T. harzianum*, Agrimm) were added to the planting furrows at 20 g m^{-1}. Control plant sites were not treated. Graftlings were planted at 5 cm spacing within rows, and 60 cm between rows. After planting, the root zones of the treated plots were drenched with *Trichoderma* (Trichogrow™, selected strains of *T. harzianum*, Agrimm) (1.5 kg ha^{-1}) at monthly intervals. A total of six applications were made. The control vines were treated with water. Normal nursery practices (irrigation, nutrition, cultural practices and disease and pest management) were followed for all the vines.

Vine growth was monitored throughout the season. The longest shoots of 27 randomly selected *Trichoderma* treated and 27 control vines were measured 5 weeks after planting. After 8 months in the nursery, the plants were uprooted and percentage take (yield of Class 1 vines as a percentage of the total number of vines planted in each plot) was determined. Twenty-five vines per replicate were randomly selected, their total root and shoot mass determined, and used for fungal isolation. Rootstock (6 cm from the basal end) and five root segments (6 cm from the basal rootstock attachment) were cut from the vines, sterilised (30 s in 70% ethanol, 2 min in 3.5% sodium hypochlorite and 30 s in 70% ethanol) and air-dried in a laminar flow cabinet. Five small (1 × 0.5 mm) sections were cut from the xylem tissue in the rootstock (approximately 3 cm from the basal end of the rootstock) and five in each root segment (3 cm from the rootstock attachment) and plated on potato dextrose agar medium (Biolab, Midrand, South Africa) amended with 250 mg l^{-1} chloramphenicol. Inoculated plates were incubated for 21 days at 23°C under a diurnal light schedule. Fungal cultures were microscopically identified. The incidence of Petri disease fungi (*P. chlamydospora* and *Phaeoacremonium* spp.), *Cylindocarpon* spp., "total number" of fungi and bacteria present in each rootstock and root was determined as a percentage of the five isolated segments colonised. Data were subjected to analysis of variance using SAS version 8.1 (SAS, 1990). Student's t-Least Significant

Difference was calculated at the 5% confidence level to compare treatment means.

Results

Five weeks after planting, shoots of *Trichoderma* treated vines were markedly longer than those of the control vines (102.6 ± 64.77 mm vs. 70.2 ± 35.91 mm). Eight months after planting, percentage take and shoot mass were not increased by the *Trichoderma* treatments, but total root mass was significantly increased from the 25.73 g of the control vines to 36.46 g (Table 1).

The incidence of various fungal genera isolated from the rootstocks and roots of the *Trichoderma* treated and control vines is given in Table 2. *Phaeomoniella* was the more prominent of the Petri disease fungi, and colonised a mean of 4.7% of the total number of xylem segments isolated from roots and rootstocks in this trial. *Phaeoacremonium* spp.

Table 1. Mean percentage take and total root and shoot masses of *Trichoderma* treated and standard treated (control) nursery grapevines.

Treatment	Percentage take	Total shoot mass (g)	Total root mass (g)
Trichoderma	43.05 ± 3.145a	20.46 ± 0.510a	36.46 ± 2.014a
Control	44.58 ± 2.863a	18.92 ± 2.044a	25.73 ± 1.638b
L.S.D.	10.407	5.154	6.352

Values in each column followed by the same letter do not differ significantly (*P*=0.05).

Table 2. Percentage of various fungal genera isolated from rootstocks and roots of *Trichoderma* treated and standard treated (control) nursery grapevines.

Fungal genus	Rootstock (%)[a]		Roots (%)[a]	
	Trichoderma	Control	*Trichoderma*	Control
Acremonium	1.8	1.8	1.8	1.4
Alternaria	0.2	0	0.2	0.2
Aspergillus	0	0.2	0	0.4
Botryosphaeria	1	0.2	0	0
Chaetophoma	0.4	0	0.4	1.4
Cylindrocarpon	0.4	0.4	1.6	2.8
Cladosporium	0.2	0	0	0.2
Fusarium	0	0.4	0.2	1
Gliocladium	0	0	0.2	0.2
Paecilomyces	0	0.2	0	0
Penicillium	0.2	1.2	3	1.2
Phaeoacremonium	1	0.8	1.2	1.4
Phaeomoniella	4.4	7.8	3.2	3.4
Phialophora	0	0	0	0.2
Phoma	13.6	2.8	19.2	26.6
Phomopsis	0.8	2.2	0	0
Pyrenochaeta	0	0	0.4	0
Rhizoctonia	0	0	0	0.2
Robillarda	0.2	0	0.2	0
Unidentified	0.6	2.8	1.2	3.6

[a] Total number of xylem segments (5 segments per vine, 25 vines per repetition, 4 repetitions) that were colonised by the fungi, expressed as a percentage of the total number of segments used for isolation in each treatment.

colonised a mean of 1.1% of the segments, and was seemingly not influenced by the *Trichoderma* treatments. Other fungal genera that include known grapevine pathogens were *Botryosphaeria*, *Phomopsis* and *Rhizoctonia* species. *Phoma* was the most frequently isolated fungal genus (15.6%).

Statistical analyses were performed for the Petri disease fungi, *Cylindrocarpon* spp., "total fungal" and "total bacterial" counts. Due to the large variance of infection levels, a statistically significant difference at the 5% confidence level was observed for the total number of bacteria isolated from the rootstocks only (Table 3). Significantly fewer bacteria were isolated from the *Trichoderma* treated vines (23.8% vs. 37.4%). The bacteria were not identified. An equal isolation percentage of "total fungi" was obtained from rootstocks of *Trichoderma* treated and from control vines (24.8 and 25.8% respectively), as was also the case with *Cylindrocarpon* spp., which occurred at relatively low percentages (0.4% for both treatments). Although the difference was not statistically significant, markedly fewer Petri disease fungi occurred in rootstocks of *Trichoderma* treated vines (5.2 vs. 8.6%). From the roots (Table 4), none of the fungal or bacterial counts proved to be significant at the 5% confidence level, but markedly fewer bacteria and fungi were isolated from the *Trichoderma* treated vines. The incidences of Petri disease fungi in the roots of *Trichoderma* treated and control vines were similar, but fewer *Cylindrocarpon* spp. occurred in the *Trichoderma* treated vines (1.6 vs. 2.8%).

Discussion

Although the growth-stimulating attributes of *Trichoderma* soil amendments on a variety of crops have been well documented (Chang *et al.*, 1986; Windham *et al.*, 1986; Chet, 1987; Harman, 2000), this is, to the knowledge of the authors, the first report of enhanced root development of grapevine resulting from *Trichoderma* soil amendments. Al-

Table 3. Mean percentages[a] of Petri disease fungi (*Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.), *Cylindocarpon* spp., total fungi and total bacteria present in rootstocks of *Trichoderma* treated and standard treated (control) nursery grapevines.

Treatment	Microorganisms (%)[a]			
	Petri disease fungi	*Cylindrocarpon*	Total fungi	Total bacteria
Trichoderma	5.2 ± 1.49a	0.4 ± 0.40a	24.8 ± 3.17a	23.8 ± 2.77a
Control	8.6 ± 2.35a	0.4 ± 0.40a	25.8 ± 3.26a	37.4 ± 3.45b
L.S.D.	6.027	1.384	17.859	6.177

[a] Mean percentage of xylem segments (5 segments per vine, 25 vines), that were colonised by fungi or bacteria. Values in each column followed by the same letter do not differ significantly (*P*=0.05).

Table 4. Mean percentages of Petri disease fungi (*P. chlamydospora* and *Phaeoacremonium* spp.), *Cylindocarpon* spp., total fungi and total bacteria present in roots of *Trichoderma* treated and standard treated (control) nursery grapevines.

Treatment	Microorganisms (%)[a]			
	Petri disease fungi	*Cylindrocarpon*	Total fungi	Total bacteria
Trichoderma	4.4 ± 1.09a	1.6 ± 0.79a	32.8 ± 3.07a	29.4 ± 2.79a
Standard	4.6 ± 1.02a	2.8 ± 0.99a	46.2 ± 3.74a	43.4 ± 3.25a
L.S.D.	5.866	3.343	29.842	18.806

[a] Mean percentage of xylem segments (five segments per vine, 25 vines), that were colonised by fungi or bacteria. Values in each column followed by the same letter do not differ significantly (*P*=0.05).

though root development was not determined at an early stage, increased root development can be anticipated from the 46.1% longer shoot growth measured 5 weeks after planting. It is common nursery practice to top vines at regular intervals to stimulate root development and prevent excessive shoot growth. As a consequence total shoot mass of the *Trichoderma* treated vines was only marginally (8.1%) better than the control vines. Nonetheless, root development of the *Trichoderma* treated vines was enhanced by 41.7%. Since a large proportion of a grapevine's reserves is stored in the roots (Hunter, 1998), this attribute would be beneficial to the vine's ability to withstand replant shock. Given the general increase in growth, it was surprising to note that the *Trichoderma* treatments did not result in better percentage take. The percentage take of both treatments was low, but nonetheless similar to what is generally experienced in South African grapevine nurseries.

However, by enhancing vine and root development, tolerance to stress would also be increased (Harman, 2000). Stress predisposes plants to attack by several pathogens, in particular, it predisposes grapevines to the Petri disease fungi, *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. (Ferreira *et al.*, 1999; Fourie and Halleen, 2001), and also to *Cylindrocarpon* spp., which causes black foot rot (Scheck *et al.*, 1998; Fourie and Halleen, 2001). Almost 40% fewer Petri disease fungi were isolated from the rootstocks and 42.9% fewer *Cylindrocarpon* spp. from the roots was isolated from *Trichoderma* treated vines. These reductions were not statistically significant, but certainly remarkable, given the quiescent nature of these pathogens in these young asymptomatic vines. Besides the increased stress tolerance of *Trichoderma* treated vines, the reduced incidence of decline pathogens can also be attributed to induced resistance. *Trichoderma* has the ability to induce resistance in several other annual crops (Harman, 2000), but this phenomenon is not often reported for a perennial crop like grapevine. However, Calderon *et al.* (1993) and De Meyer *et al.* (1998) detected induced resistance responses in grapevine following inoculation with *T. viride* and *T. harzianum,* respectively.

Furthermore, by colonising the rhizosphere, *Trichoderma* might have prevented root pathogens from attacking developing roots by antagonism or competition (Chet, 1987). The presence of *Phytophthora* and *Pythium* spp. in South African grapevine nursery soils has been shown (Marais, 1980), but because of the frequent use of phosphonates to control downy mildew in grapevine nurseries, these pathogens have not caused serious losses. Low levels of infection might however affect root reduction or slow root development. *Cylindrocarpon* spp. can also cause a reduction in root mass (Scheck *et al.*, 1998). Halleen and Crous (2001) have shown that these fungi were isolated with increasing frequency over time from roots and rootstocks of nursery grapevines. Increased root mass and general vigour, as well as the reduced incidence of *Cylindrocarpon* spp. in the roots of *Trichoderma* treated vines might therefore be attributed to biological control of these pathogens by *Trichoderma*.

Collectively, these results demonstrate the potential of *Trichoderma* treatments in grapevine nurseries for the production of stronger vines with lower *Phaeomoniella/Phaeoacremonium* and *Cylindrocarpon* infection levels.

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