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Control of black foot disease in grapevine nurseries

F. Halleen[a,b]*, P. H. Fourie[b] and P. W. Crous[b,c]

[a]*ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council), Private Bag X5026, Stellenbosch 7599;* [b]*University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa; and* [c]*Centraalbureau voor Schimmelcultures, PO Box 85167, 3508 AD Utrecht, The Netherlands*

Black foot disease of grapevines is a decline and dieback disease caused by a soilborne pathogen complex including *Cylindrocarpon liriodendri*, *C. macrodidymum*, *Campylocarpon fasciculare* and *Campyl. pseudofasciculare*. These pathogens cause primary infections of roots and basal ends of grafted cuttings in nursery soils. Thirteen fungicides were screened *in vitro* for mycelial inhibition of these pathogens. Prochloraz manganese chloride, benomyl, flusilazole and imazalil were the most effective fungicides tested, and were subsequently included in semi-commercial field trials. Basal ends of grafted cuttings were dipped in various chemical and biological treatments prior to planting in open-rooted nurseries. Black foot pathogens were not isolated from grafted cuttings prior to planting. Additional treatments involved soil amendments with *Trichoderma* formulations and hot water treatment of dormant nursery grapevines. Field trials were evaluated after eight months. Isolations from uprooted plants revealed low levels of black foot pathogens in the roots of untreated control plants, and significantly higher levels in basal ends of rootstocks. The incidence of black foot pathogens, as well as that of Petri disease pathogens, was not significantly and/or consistently reduced by the majority of chemical or biological treatments. However, these pathogens were not isolated from uprooted plants that were subjected to hot water treatment. It is therefore recommended that hot water treatment of dormant nursery plants be included in an integrated strategy for the proactive management of these diseases in grapevine nurseries.

Keywords: *Campylocarpon* spp., *Cylindrocarpon* spp., hot water treatment, Petri disease, *Phaeoacremonium* spp., *Phaeomoniella chlamydospora*

Introduction

Black foot disease, caused by species of *Cylindrocarpon* (teleomorph *Neonectria*) and *Campylocarpon*, is a relatively recently described disease affecting mainly young grapevines (*Vitis* spp.). Two species, *Cylindrocarpon obtusisporum* and *C. destructans* have previously been associated with this disease. *Cylindrocarpon obtusisporum* caused a decline of rooted grapevine cuttings in a Sicilian nursery (Grasso & Magnano Di San Lio, 1975). The decline was characterized by stunting, black discoloration of the wood and gum inclusions of xylem vessels. This species has also been associated with black foot disease in Californian vineyards (Scheck *et al*., 1998a,b). In France, the disease is caused by a different species, *C. destructans* (Maluta & Larignon, 1991) and losses of 50% or more have been recorded in young 2–8-year-old vineyards (Larignon, 1999). A third species, *C. macrodidymum*, as well as two newly described *Campylocarpon* species, *Campyl. fasciculare* and *Campyl. pseudofasciculare*, have recently been associated with the disease (Halleen *et al*., 2004). Although *Cylindrocarpon destructans* appears to be the dominant species associated with black foot disease (Halleen *et al*., 2004), recent research has revealed that this taxon is actually a species complex (Samuels & Brayford, 1990; Seifert *et al*., 2003), and that the dominant grapevine pathogen in South Africa is *C. liriodendri* (Halleen *et al*., 2006), and not *C. destructans* as reported previously (Halleen *et al*., 2004).

In spring, affected plants are characterized by the absence of budding, or delayed budding with abnormal, weak vegetation that often wilts during early summer. Roots of infected plants become necrotic brown to black. To compensate for the loss in root mass, a second crown of horizontally growing roots is sometimes formed close to the soil surface (Larignon, 1999; Fourie *et al*., 2000). In older vines, the rootstock diameter below this second crown can be thinner (Fourie & Halleen, 2001a). When bark of severely affected vines is removed, a brown necrotic lesion originating at the base of the rootstock can be seen. A cross section through this lesion reveals the development of necrosis from the bark to the pith (Larignon, 1999;

*E-mail: halleenf@arc.agric.za

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Fourie & Halleen, 2001a). Sweetingham (1983) also described the discoloration of the phloem from which *C. destructans* was isolated. Furthermore, fungal hyphae were visible in the ray cells of the phloem and younger xylem of the discolored tissue. Functional phloem was plugged with gum and xylem vessels were plugged with thick-walled tyloses or gum.

Isolation studies conducted in South African grapevine nurseries demonstrated that black foot pathogens from soils infected grafted grapevines once planted in open-rooted nurseries (Halleen *et al*., 2003). Black foot pathogens rarely occurred in rootstock propagation material prior to planting (Fourie & Halleen, 2002; Halleen *et al*., 2003). At the time of planting, the susceptible basal ends (especially the pith area) of most of the nursery cuttings are partly or even fully exposed. Callus roots also break during the planting process, resulting in small wounds susceptible to infection by soilborne pathogens (Halleen *et al*., 2003).

Environmental factors and host stress such as malnutrition, poor drainage, soil compaction, heavy crop loads on young plants, planting of vines in poorly prepared soil and improper plant holes play an important part in the development of black foot disease (Larignon, 1999; Fourie *et al*., 2000; Fourie & Halleen, 2001b; Halleen *et al*., 2004). Soil compaction and/or poor soil preparation also contribute to poor root development (J-rooting and pothole effect) (Fourie *et al*., 2000; Halleen *et al*., 2004). High temperatures during summer also play an important role in symptom expression, since the compromised root and vascular system of diseased plants would not be able to supply enough water to compensate for the high transpiration rate during periods of high temperatures (Larignon, 1999). *Cylindrocarpon* species are often part of disease complexes with other fungi or nematodes (Brayford, 1993). In the case of declining grapevines, black foot pathogens are often isolated in association with other pathogens such as *Phaeomoniella chlamydospora*, *Phaeoacremonium* (Petri disease pathogens), *Botryosphaeria*, *Phomopsis*, *Pythium* and *Phytophthora* spp. (Fourie *et al*., 2000; Fourie & Halleen, 2001c; Edwards & Pascoe, 2004; Oliveira *et al*., 2004).

Presently, no measures are known for control of black foot disease in South African vineyards or nurseries (Nel *et al*., 2003). In nurseries, Fourie & Halleen (2004, 2006) demonstrated that soaking propagation material prior to grafting in benomyl or a patented didecyldimethylammonium chloride formulation (Sporekill®, ICA International Chemicals Pty. Ltd.) were effective in limiting infection by most trunk disease pathogens in basal ends and graft unions of nursery plants. However, these treatments did not have an effect on infection by black foot pathogens, most likely due to the fact that infection by these pathogens was shown to occur from nursery soils (Halleen *et al*., 2003). Management strategies recommended for prevention and disease management in South African vineyards mainly involves the prevention and/or correction of predisposing stress situations (Fourie & Halleen, 2001a). However, knowledge pertaining to the disease cycle of black foot disease suggests that suitable management strategies should focus on prevention of primary infection in nurseries.

Rego *et al.* (2005) conducted *in vitro* fungicide screenings and found that prochloraz, benomyl, cyprodinil + fludioxonil and carbendazim + flusilazole inhibited mycelial growth of *C. liriodendri* (as *C. destructans*), whilst tebuconazole and difenoconazole were less effective. Cyprodinil + fludioxonil, azoxystrobin, tryfloxistrobin and tolyfluanide effectively reduced spore germination (Rego *et al*., 2005). Results from subsequent *in vivo* studies conducted on pre-inoculated potted grapevines in Portugal proved that benomyl, tebuconazole, carbendazim + flusilazole and cyprodinil + fludioxonil significantly improved plant growth and decreased disease incidence (Rego *et al*., 2005).

Biological control measures have also been tested in glasshouse trials with potted grapevines, and the mychorrizal fungus *Glomus intraradices* provided excellent control against black foot disease when applied preventatively (Gubler *et al*., 2004). However, none of these treatments have been tested under field conditions. In a semi-commercial nursery trial, Fourie *et al.* (2001) demonstrated the growth stimulating attributes of commercial products of *Trichoderma*, as well as the effect on natural infection by decline pathogens such as *Cylindrocarpon* spp. Low levels of *Cylindrocarpon* spp. were recorded, but the *Trichoderma* treatments notably reduced their incidence in roots of nursery grapevines. *Trichoderma* significantly improved root development, which would make plants more tolerant to black foot disease when subjected to stress (Fourie *et al*., 2001). In a study aimed at the proactive management of Petri disease in grapevine nurseries, Fourie & Halleen (2004) found that hot water treatment (30 min at 50°C) of rootstock cuttings prior to grafting or hot water treatment of dormant nursery plants after uprooting proved to be effective in reducing infection levels in nursery plants. The latter treatment appeared to eradicate black foot pathogens from rootstocks, but infection levels were unfortunately too low for this finding to be conclusive (PHF & FH, unpublished results).

In the present study, fungicides were evaluated for their ability to inhibit black foot pathogens *in vitro*. Semi-commercial nursery trials were subsequently conducted to evaluate the effectiveness of selected fungicides, *Trichoderma* treatments and hot water treatment in preventing or eradicating natural infection of the roots and basal ends of nursery grapevines. Means to prolong residual activity of fungicides were also investigated. Naturally infected material was used in the field trials. The effect of these treatments on black foot pathogens as well as Petri disease pathogens was recorded. These pathogens are regularly co-isolated from diseased grapevines (Fourie & Halleen, 2001b) and a fungicide that controls both of these disease complexes could be of great benefit to the grapevine industry, especially in nurseries where primary infection should be prevented.

Table 1 EC_{50} values for inhibiting *in vitro* mycelial growth of *Cylindrocarpon* and *Campylocarpon* spp. by fungicides representing different chemical classes

Fungicide	EC_{50} values (μg mL^{-1} a.i.)[a]				LSD ($P = 0{\cdot}05$)
	C. liriodendri	*C. macrodidymum*	*Campyl. fasciculare*	*Campyl. pseudofasciculare*	
Benomyl	0·89 b	1·06 a	0·80 b	1·11 a	0·09
Captab	87·25 a	43·27 b	81·60 a	75·49 a	17·70
Flusilazole	0·80 b	0·21 b	12·63 a	12·15 a	3·42
Hydroxyquinoline sulphate	>100	>100	>100	>100	Not Determined
Imazalil	1·53 c	0·62 c	100·00 a	61·23 b	4·04
Kresoxim-methyl	88·89 a	100·00 a	100·00 a	100·00 a	18·78
Mancozeb	62·87 b	29·33 c	51·33 b	91·76 a	15·66
Prochloraz manganese chloride	0·04 c	0·01 d	0·26 b	0·29 a	0·02
Procymidone	100·00 a	88·89 a	71·43 a	100·00 a	32·95
Propineb	71·30 a	19·77 c	47·30 b	72·51 a	5·75
Pyrimethanil	100·00 a	98·40 a	100·00 a	100·00 a	1·60
Spiroxamine	64·77 c	100·00 a	100·00 a	78·29 b	8·04
Thiram	58·01 b	24·84 c	78·64 a	54·17 b	13·51

[a]Values in the same row followed by the same letter do not differ significantly ($P = 0{\cdot}05$).

Materials and methods

In vitro evaluation of fungicides

Isolates

Cylindrocarpon liriodendri (CBS 112607, CBS 112597, CBS 112606), *C. macrodidymum* (CBS 112615, CBS 112603, CBS 112594), *Campylocarpon fasciculare* (CBS 112611, CBS 112612, CBS 112613) and *Campyl. pseudofasciculare* (CBS 112592, CBS 112679) isolates used in this study were previously isolated from symptomless grapevine nursery plants, as well as from diseased grapevines (Halleen *et al*., 2004). During this study, isolates were maintained on 2% potato dextrose agar (PDA, Biolab) slants at 4°C and transferred to PDA in Petri dishes when needed and incubated at room temperature for 1 week, at which time there was sufficient growth to transfer to fungicide-amended media.

Fungicides

Thirteen fungicides (Table 1), representing 10 different chemical classes, were screened for *in vitro* mycelial inhibition of *Cylindrocarpon liriodendri*, *C. macrodidymum*, *Campylocarpon fasciculare* and *Campyl. pseudofasciculare*. The fungicides were: thiram (Thiram® 750WP, Dow Agrosciences); benomyl (Benomyl® 500WP, Dow Agrosciences); flusilazole (Olymp® 100EW, Du Pont de Nemours International Societe Anonyme); mancozeb (Penncozeb® 750WG, Du Pont); kresoxim-methyl (Stroby® 500WG, BASF); spiroxamine (Prosper® 500EC, Bayer); pyrimethanil (Scala® 400SC, Bayer); propineb (Antracol® 70W, Bayer); prochloraz manganese chloride (Octave® 500WP, Bayer); procymidone (Sumisclex® 250SC, Philagro); captab (Kaptan® 500WP, Applied Chemical Products); hydroxyquinoline sulphate (Chinosol 67% a.i., Algro-Chem) and imazalil (Magnate® 800EC, Makhteshim). The fungicides were suspended in sterile distilled water and added to molten PDA at approximately 50°C in amounts to achieve final concentrations of 0, 0·01, 0·05, 0·1, 0·5, 1, 5 and 10 a.i. μg mL^{-1}. Mycelial plugs (4·5 mm in diameter), obtained from the margins of actively growing cultures, were transferred to fungicide amended plates. Three plugs comprising three different isolates were placed at an equal distance from each other on each plate. There were three replicates of each fungicide concentration, and the experiment was repeated once. Concentrations of 50 and 100 μg mL^{-1} were added for less effective fungicides when the experiment was repeated. The dishes were incubated for 6 days at 23°C in the dark, and the diameter of each colony was measured twice perpendiculary.

Field trials

Field trials were conducted at two nurseries in Wellington during the 2002–2003 and 2003–2004 seasons. In total, 4825 Cabernet Sauvignon (clone CS163B)/101–14 Mgt (clone AA26A) cuttings were hand grafted (September, 2002 and 2003). All grafting took place at the same nursery according to standard nursery practices. None of the plants received any treatment at this stage. Graftlings were placed in boxes (300 per box) filled with moist sawdust for standard cold callusing.

Treatments

All 4825 graftlings were removed from callusing boxes on either 10 October 2002 or 21 October 2003. Basal ends (bottom 5–10 cm) of the rootstocks were dipped for 1 min in various treatments (300 grafted cuttings per treatment). The treatments were: benomyl (1 g L^{-1}); flusilazole (0·5 mL L^{-1}); prochloraz manganese chloride (1 g L^{-1}); imazalil (1·32 mL L^{-1}); Trichoflow-T™ (selected strains of *Trichoderma harzianum*, Agrimm Technologies; 2 g L^{-1}); carnauba wax (liquid wax derived from palm trees that is used in the citrus industry as a fruit wax and as carrier for

fungicides); prochloraz manganese chloride (3 g L^{-1}) + carnauba wax (1 L); benomyl (3 g L^{-1}) + carnauba wax (1 L); flusilazole (1·5 mL L^{-1}) + carnauba wax (1 L); Nu-Film 17 (an organic compound derived from Pinolene, a natural pine resin, used as a sticker, spreader and extender; Miller Chemical); 0·25 mL L^{-1}); benomyl (3 g L^{-1}) + Nu-Film 17 (0·25 mL L^{-1}); flusilazole (1·5 mL L^{-1}) + Nu-Film 17 (0·25 mL L^{-1}); and prochloraz manganese chloride (3 g L^{-1}) + Nu-Film 17 (0·25 mL L^{-1}). Control plants were dipped in water. Treated graftlings were allowed to air dry on a dripping rack. Graftlings from the various treatments were divided into two groups of 150 each and taken to the nurseries. Graftlings from the various treatments were further divided into three groups of 50 each. The trial layout was a randomized block design with two sites, three blocks and 16 treatments. Graftlings were planted at 5 cm spacing within rows, and 60 cm between rows. Normal nursery practices (irrigation, nutrition, cultivation practices and disease and pest management) were followed for the duration of each growing season.

Additional treatments involved soil amendments with *Trichoderma* formulations and hot water treatment of dormant nursery grapevines. For the soil amendment treatment Trichopel-R™ (selected strains of *T. harzianum*, Agrimm Technologies) was added to the planting furrows at 20 g m^{-1}. After planting, the root zones were drenched with Trichogrow™ (selected strains of *T. harzianum*, Agrimm Technologies) at monthly intervals (1·5 kg ha^{-1}), with a total of six applications. Dormant vines were subjected to hot water treatment (50°C for 30 min followed by 30 min in cold water) after uprooting. These plants did not receive any treatment before planting.

Infection ratings

Isolations were also made from grafted cuttings prior to planting, to determine whether rootstocks were infected with black foot and/or Petri disease pathogens. After callusing, prior to planting, 25 graftlings were taken to the laboratory where isolations were made from the rootstock (within 1–3 cm of the basal end). The basal end section was removed and surface sterilized (30 s in 70% ethanol, 5 min in 0·35% sodium hypochlorite and 30 s in 70% ethanol) before isolations were made. These sections were split lengthwise to reveal the xylem and pith regions. Four pieces of tissue (approximately 0·5 × 1 mm in size) were removed and transferred to PDA amended with chloramphenicol (250 mg L^{-1}) to reduce bacterial growth and incubated in an incubation growth room at ±25°C for 4 weeks (12 h fluorescent white light/dark regime). Subsequent fungal growth was monitored daily, identified and hyphal-tips were transferred to PDA slants for later identification.

Growth of nursery plants was monitored throughout the season. The nursery plants were uprooted after 7 months, classed by the respective nurseries (according to SA Plant Improvement Regulations), and the certifiable plant yield determined (number of Class 1 certified vines as a percentage of grafted cuttings planted). Twenty-five vines per replicate were randomly selected and total root and shoot mass of each determined, followed by fungal isolation as described above. The isolations determined the level of natural infection by black foot and other grapevine pathogens in the two nurseries. The presence of *Trichoderma* spp. was also recorded to provide an indication of the extent of colonization following treatment with the *Trichoderma* formulations. Isolations were made from the rootstock (within 1–3 cm of the basal end) as well as from the roots (within 1 cm from the basal end of the rootstock).

Statistical analyses

For *in vitro* evaluation of fungicides, percentage inhibition for each isolate at each concentration was calculated as a percentage of the control treatment (unamended PDA). The data for percentage inhibition were pooled and linear regressions were fitted over the natural log concentrations for each isolate and fungicide separately. For more accurate estimations of the EC_{50} values (concentration at which 50% of the mycelial growth was inhibited), a linear regression equation [% Inhibition = $a + b \times$ Ln(Concentration), where a = intercept and b = slope] was fitted on two points in the log regression that included the 50% inhibition value. EC_{50} values were calculated as follows: $EC_{50} = \exp(b/(50 - a))$. The Shapiro-Wilk test was performed to test normality on residuals (Shapiro & Wilk, 1965). Isolates with outliers were discarded until the residuals were normally distributed.

For field trials, experimental blocks and nurseries were combined as six blocks and the repeated measurements over seasons as a subplot factor. The incidence of fungi present in each rootstock and root was determined as a percentage of the four isolated segments colonized.

Data from the above-mentioned trials were subjected to analyses of variance using SAS version 8·1 (SAS, 1990). Student's *t*-Least Significant Difference values were calculated at the 5% confidence level to facilitate comparison between treatment means.

Results

In vitro evaluation of fungicides

Significant fungicide × species interaction ($P < 0·0001$) was observed. Mean EC_{50} values for reduction in mycelial growth of *C. destructans*, *C. macrodidymum*, *Campyl. fasciculare* and *Campyl. pseudofasciculare* are given in Table 1. Prochloraz manganese chloride was the most effective in reducing mycelial growth, with lowest EC_{50} values (0·01–0·29 μg mL^{-1}) for all four species. Benomyl was the only other fungicide that reduced mycelial growth of all four species (0·8–1·1 μg mL^{-1}). Flusilazole and imazalil were effective in reducing mycelial growth of the *Cylindrocarpon* spp. (0·2–0·8 μg mL^{-1} for flusilazole and 0·6–1·5 μg mL^{-1} for imazalil), but not the *Campylocarpon* spp. (12·2–12·6 μg mL^{-1} for flusilazole and 61·2–100 μg mL^{-1} for imazalil). All the other fungicides were significantly less effective in inhibiting mycelial growth,

Table 2 Mean incidences of fungal pathogens associated with black foot disease isolated from rootstocks and roots of grapevine nursery plants treated with various chemical and biological control agents prior to planting, as well as hot water treatment of dormant plants after uprooting

	Rootstock[b]		Roots[b]
Treatment[a]	2002–2003	2003–2004	Combined
Benomyl	40·7 bcde	12·0 gh	5·2 b
Benomyl /Carnauba wax	15·3 gh	6·9 ghi	4·6 b
Benomyl/Nu-Film 17	29·3 ef	4·6 hi	4·2 b
Carnauba wax	38·0 cde	11·4 ghi	4·0 b
Control	45·3 abc	16·8 g	4·1 b
Control (isolations before planting)	0·0 i	0·0 i	–
Flusilazole	54·0 a	15·2 gh	8·3 a
Flusilazole/Nu-Film 17	48·7 abc	10·4 ghi	4·7 b
Flusilazole/Carnauba wax	41·3 bcd	8·7 ghi	3·3 b
Hot Water Treatment	0·0 i	0·0 i	0·0 c
Imazalil	31·3 de	16·7 g	5·3 ab
Nu-Film 17	54·7 a	15·7 gh	3·1 b
Prochloraz manganese chloride	53·3 a	12·2 gh	4·3 b
Prochloraz manganese chloride/Carnauba wax	45·0 abc	10·0 ghi	4·7 b
Prochloraz manganese chloride/Nu-Film 17	49·3 abc	11·3 ghi	4·0 b
Trichoflow	40·7 bcde	10·7 ghi	3·7 b
Trichopel/Trichogrow	50·0 ab	18·1 fg	4·2 b
LSD ($P = 0·05$)	11·67		2·97

[a]Graftlings were treated on 10 October 2002 and 21 October 2003 by dipping the basal ends of the graftlings in various treatments for 1 minute.
[b]Percentage incidence was determined by means of isolations after uprooting in May–June 2003 and 2004. Values within the respective columns followed by the same letter do not differ significantly ($P = 0·05$).

with EC_{50} values ranging from 19·8 to > 100 μg mL^{-1}. Based on these results, prochloraz manganese chloride, benomyl, flusilazole and imazalil were selected to be included in nursery trials for further evaluation.

Field trials

Certifiable plant yield

Analysis of variance of certifiable plant yield percentages indicated that no significant interaction was observed between season and treatment ($P = 0·0710$; ANOVA not shown). Certifiable plant yields were significantly ($P < 0·0001$) higher during the 2002–2003 season (78·4%) compared to that in 2003–2004 (71·3%). Significant differences were furthermore observed between means for treatments ($P < 0·0001$). The prochloraz manganese chloride/Nu-Film 17 (73·3%), flusilazole/Nu-Film 17 (70·4%), carnauba wax (68·0%), flusilazole/carnauba wax (68·0%) and prochloraz manganese chloride (66·7%) treatments caused reductions of 7·6% to 15·9% in certifiable plant yield compared to the water treated control plants (79·3%). None of the other treatments (73·5% to 84·0%) differed significantly from the water treated control plants (results not shown).

Root and shoot mass

Analyses of variance for root and shoot mass data indicated significant differences between seasons ($P = 0·0331$ and $P < 0·0001$, respectively). However, no significant treatment × season interaction was observed for root or shoot mass ($P = 0·8071$ and $P = 0·8577$, respectively). None of the treatments yielded plants with roots or shoots lower in mass than the water treated controls. However, the carnauba wax treated plants had significantly heavier roots (mean of 35·1 g) than the water control (29·2 g), whereas the prochloraz manganese chloride (26·5 g), benomyl/carnauba wax (26·6 g), carnauba wax (28·3 g) and flusilazole/Nu-Film 17 (28·4 g) treated plants had significantly heavier shoots than the water treated control (22·8 g; results not shown).

Incidence of black foot pathogens

Significant treatment × season interaction ($P < 0·0001$) occurred for the mean incidence of black foot pathogens in rootstocks, and datasets for the two seasons could therefore not be combined. No black foot pathogens were isolated from any of the control plants prior to planting in the nurseries (Table 2), but infection levels in the water treated control plants were significantly higher at the end of each growing season, and significantly higher during the 2002–2003 season (45·3%) compared to the 2003–2004 season (16·8%). Imazalil (incidence of black foot pathogens, 31·3%), benomyl/Nu-Film 17 (29·3%), benomyl/carnauba wax (15·3%) and hot water treatment (0·0%) were the only treatments that differed significantly from the water treated control (45·3%) in the 2002–2003 season, whilst only the benomyl/Nu-Film 17 (4·6%) and hot water treatment (0·0%) harboured significantly lower levels of black foot pathogens compared to the control (16·8%) in the 2003–2004 season.

In roots, no significant interaction was observed between season and treatment ($P = 0·3725$) and the datasets of the

Table 3 Mean incidences of Petri disease pathogens isolated from rootstocks and roots of grapevine nursery plants treated with various chemical and biological control agents prior to planting, as well as hot water treatment of dormant plants after uprooting

	Phaeomoniella chlamydospora[b]				*Phaeoacremonium* spp.[b]		
	Rootstock		Roots		Rootstock	Roots	
Treatment[a]	2002–2003	2003–2004	2002–2003	2003–2004	Combined data for both seasons	2002–2003	2003–2004
Benomyl	4·0 efgh	5·3 defgh	0·0 d	1·5 bcd	3·3 cde	2·7 efgh	3·3 efgh
Benomyl/Carnauba wax	10·7 cd	2·0 fgh	1·3 bcd	0·0 d	2·5 de	6·7 bcdef	6·6 bcdef
Benomyl/Nu-Film 17	10·0 cde	4·3 efgh	0·0 d	0·0 d	2·0 de	6·7 bcdef	1·3 gh
Carnauba wax	2·0 fgh	10·7 cd	0·0 d	2·7 ab	2·7 cde	10·7 b	6·8 bcdef
Control	5·3 defgh	21·7 a	0·7 cd	3·5 a	6·0 abcd	6·0 bcdefg	5·3 cdefg
Control (isolations before planting)	0·0 h	0·0 h	.	.	0·0 e	.	.
Flusilazole	6·0 defgh	15·3 abc	0·0 d	1·3 bcd	5·3 abcd	7·3 bcde	6·0 bcdefg
Flusilazole/Carnauba wax	5·3 defgh	4·0 efgh	0·0 d	2·0 abc	5·3 abcd	18·0 a	2·7 efgh
Flusilazole /Nu-Film 17	9·3 cde	6·4 defg	0·0 d	0·0 d	4·7 abcd	6·0 bcdefg	3·2 efgh
Hot Water Treatment	0·0 h	0·0 h	0·0 d	0·0 d	0·3 e	0·0 h	0·0 h
Imazalil	6·7 defg	2·0 fgh	0·0 d	0·0 d	4·0 bcde	9·3 bc	2·7 efgh
Nu-Film 17	2·1 fgh	10·2 cde	0·7 cd	2·0 abc	4·6 abcd	6·1 bcdefg	3·3 efgh
Prochloraz manganese chloride (PMC)	0·7 gh	7·1 def	0·0 d	0·7 cd	8·1 a	5·3 cdefg	3·5 defgh
PMC/Carnauba wax	4·7 defgh	5·3 defgh	0·0 d	0·0 d	7·7 ab	6·9 bcdef	8·7 bcd
PMC/Nu-Film 17	5·3 defgh	6·7 defg	0·0 d	1·3 bcd	6·7 abc	4·7 cdefgh	5·3 cdefg
Trichoflow	6·7 defg	18·0 ab	0·0 d	1·3 bcd	2·3 de	6·0 bcdefg	1·3 gh
Trichopel/Trichogrow	4·0 efgh	7·5 def	0·0 d	0·0 d	2·3 de	2·0 fgh	2·0 fgh
LSD ($P = 0·05$)	6·19		1·63		4·00	5·26	

[a]Graftlings were treated on 10 October 2002 and 21 October 2003 by dipping the basal ends of the graftlings in various treatments for 1 minute.
[b]Percentage incidence was determined by means of isolations after uprooting in May–June 2003 and 2004. Values within the respective columns followed by the same letter do not differ significantly ($P = 0·05$).

two seasons were combined. Significant differences ($P < 0·0001$) were observed between seasons and contrary to what was observed in rootstocks, significantly lower incidences were observed in the 2002–2003 season (2·8%) compared to the 2003–2004 season (5·7%). Significant differences ($P = 0·0067$) were also observed between the treatments. Isolations made from uprooted grapevines at the end of the growing season revealed very low levels (mean incidence of 4·1%) of black foot pathogens in the water treated control plants (Table 2). Significantly more black foot pathogens were isolated from roots of flusilazole treated plants (8·3%) compared to the control plants. No black foot pathogens were isolated from roots of plants that were subjected to hot water treatment, while none of the other treatments differed significantly from the control plants.

Incidence of Petri disease pathogens

Significant treatment × season interaction ($P < 0·0001$) was observed for the incidence of *Phaeomoniella chlamydospora* in rootstocks. The fungus was not isolated from any of the control plants prior to planting (Table 3). However, higher levels were recorded from the water treated control plants at the end of the growing season, with significantly more in the 2003–2004 season (incidence of Petri disease pathogens, 21·7%) than the 2002–2003 season (5·3%). All the treatments (mean incidence of 0·0–10·7%), except Trichoflow-T™ (18·0%) and flusilazole (15·3%), reduced infection during the 2003–2004 season compared to the water treated control. None of the treatments differed from the water treated control during the 2002–2003 season. However, it should be noted that *P. chlamydospora* was not isolated from hot water treated plants in any of the seasons. In roots, significant treatment × season interaction was also observed ($P = 0·0355$). *Phaeomoniella chlamydospora* levels in roots of control plants were approximately 7-fold less than those observed for rootstocks (Table 3). None of the treatments differed significantly from the water treated control during the 2002–2003 season. However, all the treatments except carnauba wax (2·7%), flusilazole/carnauba wax (2·0%) and Nu-Film 17 (2·0%) reduced infection during the 2003–2004 season compared to the water treated control (3·5%; Table 3). As in rootstocks, *P. chlamydospora* was not isolated from roots of hot water treated plants (Table 3).

For *Phaeoacremonium* spp. isolated from rootstocks, treatment × season interaction was not significant ($P = 0·1586$). Contrary to what was observed for *P. chlamydospora*, significantly ($P = 0·0239$) less *Phaeoacremonium* spp. were isolated from rootstocks during the 2003–2004 season (mean incidence of 3·2%) compared to the 2002–2003 season (4·8%). Significant differences ($P < 0·0014$) were also observed between the treatments. No *Phaeoacremonium* spp. were isolated from any of the control plants prior to planting in the nurseries, whilst these species were isolated from the basal ends of 6% of the water treated control plants after uprooting (Table 3). None of

the other treatments, except for the hot water treatment (0·3%), differed significantly from the water treated control. In roots, significant treatment × season interaction was observed ($P = 0{\cdot}0050$). *Phaeoacremonium* spp. were not isolated from the roots of plants that were subjected to hot water treatment, which was the only treatment that reduced the incidence of *Phaeoacremonium* spp. compared to the control plants during both seasons (6·0 and 5·3%, respectively; Table 3). The flusilazole/carnauba wax treatment (18%) caused an increase in *Phaeoacremonium* incidence during the 2002–2003 season, whereas it effected a reduction during 2003–2004 (2·7%; Table 3).

Incidence of Trichoderma *spp.*

No interaction was observed between season and treatment ($P = 0{\cdot}1098$). Significantly higher *Trichoderma* incidences ($P = 0{\cdot}0022$) were observed during the 2002–2003 season (mean of 1%) compared to the 2003–2004 season (0·3%). Significant differences ($P = 0{\cdot}0052$) were also observed between the treatments. *Trichoderma* spp. were not isolated from any of the control plants prior to planting in the nurseries. Isolations from uprooted grapevines revealed very low levels (0·3%) of *Trichoderma* spp. in the basal ends of water treated control plants. Only the Trichoflow-T™ and prochloraz manganese chloride/carnauba wax treatments differed from the water treated control plants (2·3% and 1·8%, respectively). In roots, significant treatment × season interaction occurred ($P = 0{\cdot}0208$). *Trichoderma* spp. were not isolated from any roots in 2002–2003, while flusilazole/Nu-Film 17 treatment (3·2%) was the only treatment that differed from the water treated control (0%) during the 2003–2004 season. *Trichoderma* spp. were not isolated from roots of plants subjected to *Trichoderma* treatments during both seasons.

Discussion

Results from the *in vitro* studies indicated that prochloraz manganese chloride and benomyl were the most effective in inhibiting mycelial growth of the *Cylindrocarpon* and *Campylocarpon* spp. tested, while flusilazole and imazalil were highly effective against the *Cylindrocarpon* spp. only. This is the first report of *in vitro* fungicide sensitivity of *C. macrodidymum*, *Campyl. fasciculare* and *Campyl. pseudofasciculare*, while the findings support those reported for *C. liriodendri*/*C. destructans* by Rego *et al.* (2005). Benomyl is registered for the control of grapevine powdery mildew (*Erysiphe necator*) and botrytis rot (*Botrytis cinerea*) and flusilazole for the control of grapevine powdery mildew (Nel *et al.*, 2003). Prochloraz manganese chloride is registered in South Africa for use on apricots, peaches, plums, roses, mushrooms and potatoes (Nel *et al.*, 2003). Imazalil is registered for the control of post harvest decay on citrus and cucurbits. On citrus it is generally applied in wax, or in water before waxing of fruit, as a spray-on or brush-on application (Nel *et al.*, 2003). However, the latter two compounds are not registered for use on grapevines. Previous studies have shown that prochloraz manganese chloride, benomyl and flusilazole also effectively inhibited mycelial growth of *P. chlamydospora* (Groenewald *et al.*, 2000; Jaspers, 2001), whereas prochloraz manganese chloride and benomyl also inhibited spore germination (Jaspers, 2001).

The differences in root and shoot mass between seasons might be attributed to climatic differences between seasons and/or differences in nursery practices. The increase in root and shoot mass observed in some treatments might be due to the low certifiable plant yields of the respective treatments, which results in reduced competition between plants (Fourie & Halleen, 2006). However, the common nursery practice where shoots are regularly topped should also be a factor.

The fact that black foot pathogens infect grapevine cuttings from nursery soils (Halleen *et al.*, 2003) has been confirmed in this study, and clearly underlines the importance of suitable control measures to prevent or eradicate these infections. In this study, various chemical and biological pre-planting treatments for prevention of infection by black foot and Petri disease pathogens were inconsistent, perhaps because of generally low and varying infection levels in the roots and rootstocks, respectively. The benomyl/Nu-Film 17 and benomyl/carnauba wax treatments can nonetheless be identified for further investigation, since they showed marginal to significant reductions in the incidences of these pathogens in rootstocks and/or roots. The poor performance of the various fungicides might be due to the inability of the fungicides to adequately penetrate the xylem tissue and/or breakdown of fungicide residue on the rootstock surface will also result in a limited period of protection. Prochloraz manganese chloride and imazalil were observed to be ineffective in this study, in contrast to their positive effects observed by Kuck *et al.* (1995) on various seed- and soilborne pathogens. Although registered in various countries for use as soil drenches and seed treatments, they nonetheless proved ineffective here. The addition of Nu-film 17 and carnauba wax to improve adhesion and prolong efficacy of the fungicide treatments was largely ineffective. However, the addition of these products to benomyl did lead to a marked improvement of the fungicide's efficacy.

In plants grown at two different nursery fields during the 2003/2004 season, a difference was observed in *P. chlamydospora* incidence (30% and 13·3%; results not shown). Since rootstock and scion cuttings were obtained from the same source, this difference can only be attributed to varying cultivation practices and environmental conditions between sites, or to infections from nursery soils (Crous *et al.*, 1996; Adalat *et al.*, 2000; Whiteman *et al.*, 2004; Damm & Fourie, 2005; Gaforio *et al.*, 2005; Retief *et al.*, 2006).

The *Trichoderma* treatments proved inefficient or at most inconsistent, as they prevented infection of *Phaeomoniella* in one season only (Trichopel™/Trichogrow™ treatment in 2003/2004). A possible reason might have been insufficient systemic colonization of the basal ends of rootstocks, as was shown by the low re-isolation percentages observed for *Trichoderma* spp. in plants treated with this biological control agent. In this regard, the Trichoflow™

treatment prior to planting proved to be more effective than the Trichopel-R™/Trichogrow™ treatment, although *Trichoderma* was isolated from a mere 2·3% of rootstocks. It is therefore recommended that the duration of this treatment (1 min dip) be re-evaluated in an attempt to improve colonization and prolonged protection. In previous studies these treatments reduced the incidence of *Cylindrocarpon* spp. in nursery grapevines and significantly improved root development (Fourie *et al*., 2001). Although it was not observed in this study, the growth stimulating effect would possibly make plants more tolerant when subjected to stress.

The reduction in black foot and Petri disease pathogens caused by the hot water treatment clearly demonstrated the potential of this control measure to eradicate pathogen infections from dormant nursery vines. Previously this treatment was also recommended for the eradication of *Phytophthora cinnamomi* (Von Broembsen & Marais, 1978), *Phaeomoniella chlamydospora* (Crous *et al*., 2001; Fourie & Halleen, 2004) and *Meloidogyne javanica* (Barbercheck, 1986) from dormant nursery grapevines. It is therefore recommended that hot water treatment of dormant nursery grapevines is included in an integrated strategy for the proactive management of diseases and pests in grapevine nurseries.

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