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Chemical and biological protection of grapevine propagation material from trunk disease pathogens

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Abstract

Wound protection during all stages of grapevine propagation is of utmost importance to prevent infection of propagation material by decline and dieback pathogens. In semi-commercial nursery trials, grapevine rootstock and scion cuttings were soaked in water (control), chemical or biological sanitation products prior to cold storage, prior to grafting (machine- or hand-grafting) and prior to planting in field nurseries. Natural infection levels in basal ends and graft unions of uprooted nursery grapevines were evaluated 8 months after planting. Total pathogen incidences in the water-treated control plants ranged from 30% in basal ends to 13.5% in graft unions. *Phaeomoniella chlamydospora* was the most commonly isolated pathogen, followed by *Phaeoacremonium*, *Cylindrocarpon* + *Campylocarpon*, *Botryosphaeria* and *Phomopsis* spp. Machine-grafted unions generally had lower pathogen incidences compared with hand-grafted graft unions. In general, repeated soak-treatments of propagation material in the tested products resulted in reduced pathogen incidences in nursery grapevines. However, products containing *T. harzianum* (Trichoflow-T), hydrogen peroxide (Bio-sterilizer) and 8-hydroxyquinoline sulphate (Chinosol) gave inconsistent results, whereas Bronocide (a blend of halogenated alcohols and water) proved to be a good sterilising agent, but reduced certifiable plant yield significantly. Benomyl (at 100 g/100 l), Sporekill (a patented didecyldimethylammonium chloride formulation at 150 ml/100 l) and captan (at 1000 ml/100 l) were consistently the best treatments as growth parameters were not negatively influenced and pathogen incidences in basal ends and graft unions of uprooted plants were reduced.

Introduction

Phaeomoniella chlamydospora and *Phaeoacremonium* spp. (causal organisms of Petri disease and esca) (Mugnai et al., 1999; Mostert et al., 2005), *Botryosphaeria* (Phillips, 1998, 2000, 2002; van Niekerk et al., 2004, 2005a), *Phomopsis* spp. (van Niekerk et al., 2005b) (causal organisms of grapevine dieback), and *Cylindrocarpon* and *Campylocarpon* spp. (causal organisms of black foot) (Halleen et al., 2004, 2005b) were recently described as important trunk disease causing pathogens that cause premature decline and dieback of grapevines. Recent reports have indicated that infection by these pathogens can occur as early as the propagation stages. *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. can infect rootstock mother vines and disseminate

from these infections by means of conidia or hyphal fragments via xylem vessels into the rootstock canes (Feliciano and Gubler, 2001; Ridgway et al., 2002; Edwards et al., 2003; Fourie and Halleen, 2002, 2004a). *Botryosphaeria* and *Phomopsis* spp. were also shown to infect rootstock mother plants via unprotected pruning wounds (Fourie and Halleen, 2004a) from where *Botryosphaeria* was able to infect rootstock cuttings (Fourie and Halleen, 2002). Furthermore, unprotected wounds might also be infected at the various nursery stages. *Phaeomoniella chlamydospora* DNA was detected in hydration and drench water, callus media and soils in New Zealand and South African nurseries (Whiteman et al., 2002, 2003; Damm and Fourie, 2005; Retief et al., 2005, 2006) indicating that these media should be considered as potential inoculum sources for this pathogen. *Botryosphaeria* and *Phomopsis* spp. have also been isolated from infected graft unions (Rumbos and Rumbou, 2001; Phillips, 2002). Halleen et al. (2003) also clearly demonstrated that the black foot pathogens, *Cylindrocarpon* and *Campylocarpon* spp., infected basal ends of grafted cuttings from soils in field nurseries.

The grapevine propagation process starts with the planting and maintenance of rootstock and scion mother blocks. Rootstock canes are harvested during the autumn to early winter period; cuttings are prepared, subjected to a *circa* 12-h hydration period and cold stored (1–2 °C) until grafting during late winter to early spring (Le Roux, 1988a). Before grafting, cuttings may again be soaked in water for 2–4 h (Le Roux, 1988b). In South Africa, grapevines are grafted mainly by means of long whip hand-grafting, and to a lesser extent omega-cut machine grafting (Le Roux, 1988b). Following grafting, the graft unions are dipped in a melted (80–85 °C) fungicide-impregnated wax formulation such as Graftseal (Dalven Products, Eppindust, South Africa) that contains 1 g kg^{-1} 8-hydroxyquinoline and 0.044 g kg^{-1} 2,5-dichlorobenzoic acid methyl ester, and packed in callus boxes with fresh pine sawdust. Hand-grafted cuttings are cold-callused at *circa* 18 °C for a period up to 5 weeks, while machine-grafted cuttings are hot-callused at 26–28°C and 70% RH for a period of up to 3 weeks followed by a hardening-off period of 1–2 weeks under shade netting. Following successful callusing, graftlings are transplanted in field nurseries at 5 cm in-row spacing and *circa* 60 cm between rows. Upon planting, graft unions are covered with soil, which is later removed following successful bud burst. Growing plants are frequently irrigated, mostly by means of overhead irrigation. During the 8-month field nursery period, foliar diseases, such as downy and powdery mildew, are managed by means of regular fungicide applications. Dormant nursery plants are uprooted in autumn, and either cold stored or heeled-in until replanting in spring. Plants are graded according to the standards set by the Vine Improvement Association (VIA, Box 166, Paarl 7622, South Africa), and the yield of certifiable plants (Class 1) calculated as a percentage of the number of grafted cuttings that were planted. Generally low certifiable plant yields in South Africa (5-year average of *circa* 45%; VIA, South Africa) are attributed to failed graft unions, sub-standard growth parameters, and fungal diseases, such as *Phaeomoniella chlamydospora* and *Sclerotium rolfsii* (Keyer and Ferreira, 1988).

Current practices in nurseries aimed at limiting fungal infections on woody tissues include mainly drenches or dips in contact fungicides, such as captan, 8-hydroxyquinoline sulphate, iprodione, procymidone or triadimefon (Marais and Van der Westhuizen, 1978, 1979; Le Roux, 1988a, b). However, the use of these compounds is aimed largely at limiting superficial fungal growth on propagation material during the storage and callusing stages. Moreover, these products were shown not to be highly effective against trunk disease pathogens, such as *Phaeomoniella chlamydospora* (Groenewald et al., 2000; Jaspers, 2001), *Botryosphaeria* (Bester and Fourie, 2005), *Campylocarpon* and *Cylindrocarpon* spp. (Halleen et al., 2005a; Rego et al., 2005). From these studies, benomyl was identified as a highly effective fungicide against these pathogens.

On the basis of recent research (Fourie et al., 2001; Fourie and Halleen, 2001, 2004a, b, 2005), various proactive management strategies have been recommended for prevention of infection of propagation material by trunk disease pathogens during the grafting and nursery stages. Firstly, prevention of pruning wound infection, as well as sanitation to reduce inoculum sources were recommended in mother blocks (Fourie and Halleen, 2004a). Prior to grafting, rootstock cuttings can also be soak-treated (1 h) in benomyl or *Tricho-*

derma formulations; a practice that resulted in significantly reduced *Phaeomoniella chlamydospora* and *Phaeoacremonium* infection levels in the nursery plants (Fourie and Halleen, 2004b). In this study, hot water treatment (HWT; 50 °C for 30 min) of rootstock cuttings or dormant nursery plants was shown to be the most effective treatment, as it almost completely eradicated *Phaeomoniella chlamydospora* and *Phaeoacremonium* from the cuttings and plants. Moreover, HWT of rootstock cuttings also prevented subsequent colonisation of the plants in the field nursery. HWT of dormant nursery plants was also effective in eradicating *Phytophthora cinnamomi* (von Broembsen and Marais, 1978), *Cylindrocarpon* and *Campylocarpon* spp. (Halleen et al., 2005a, b) and *Meloidogyne javanica* (Barbercheck, 1986). Despite these benefits, HWT of dormant nursery plants was not embraced as a standard treatment in South African nurseries. This is largely attributed to the logistics involved in treating large numbers of plants, as well as the increased susceptibility of the treated plants to desiccation and potentially compromised replant success. HWT of cuttings prior to grafting is more widely accepted given its practicality and the lower risk associated with this treatment. However, wound protection subsequent to this treatment is of utmost importance. For these reasons, it was therefore necessary to investigate complementary or alternative methods of limiting infection of wounds during the grafting stages.

The aim of this study was therefore to conduct a semi-commercial trial to compare the efficacy of repeated soak treatments with benomyl, *Trichoderma harzianum*, broad-spectrum fungicides and sterilising agents to prevent natural infection of basal ends and graft unions by trunk disease pathogens in grapevine nurseries. As grafting technique (hand- vs. machine-grafting) might also play a role in graft wound infection, it was studied as a sub-treatment.

Materials and methods

Experimental layout

Trial layout was a randomised block design with 4 blocks, 8×2 treatments (soak treatments and grafting regimes, respectively), and 50 grafted cuttings per treatment per block. The trial was repeated in the following season.

Soak treatments

Rootstock cuttings (101-14 Mgt; clone AA219A), scion cuttings (Shiraz; clone SH5C) were treated after cuttings were prepared from mother block canes, prior to cold storage. This treatment involved a 1-h soak in 50-l suspensions of one of the following eight products: (1) benomyl (Benlate 500WP at 100 g per 100 l water), (2) *Trichoderma harzianum* (Trichoflow-T, Agrimm Technologies Ltd., Christchurch, New Zealand; 200 g/100 l water), (3) captan (Merpan 500 SC at 1000 ml/100 l water; note that this is five times the registered dosage as recommended for control of grapevine anthracnose Nel et al., 2003), (4) a blend of halogenated alcohols and water (Bronocide SP General Purpose Disinfectant, Essential Medicines, Richards Bay, South Africa; this sanitation product is frequently used in hospitals, and since it is not phytotoxic and reasonably environmentally safe, it is also used during all aspects of citrus tissue culture and grafting; 10 l/100 l water), (5) a patented didecyldimethylammonium chloride formulation (Sporekill, ICA International Chemicals Pty.Ltd., Stellenbosch, South Africa; 150 ml per 100 l water), (6) hydrogen peroxide (Bio-sterilizer, Agro-Organics, Strand, South Africa, 1 l/100 l water), (7) 8-hydroxyquinoline sulphate (Chinosol, 67% a.i., Algro-Chem, Rynfield, South Africa; 250 g/100 l water), and (8) clean tap water as the control. One day prior to grafting, following the standard cold storage period of cuttings, they were again treated for 10 min in the above-mentioned solutions. A final dip treatment (5 s submersion) was conducted after the callusing period, prior to planting.

Grafting regimes

Grafting was conducted according to two grafting regimes (Le Roux, 1988b): machine-grafting (omega-cut grafted cuttings that are hot-callused) and hand-grafting (long-whip grafting cut; grafted cuttings are cold-callused). In each case, grafting was performed in commercial nurseries according to standard practices.

Determination of growth parameters and infection levels

The treated and grafted rootstocks were planted in a field nursery in Wellington (50 grafted cuttings per treatment per block). At the end of the growing season, 8 months after planting, the plants were uprooted. For each treatment combination, the plants were graded according to the VIA standards and the yield of certifiable plants (Class 1) calculated as a percentage of the number of grafted cuttings that were planted. From these plants, 20 were randomly selected and taken to the laboratory. Root and shoot mass of each plant was determined.

Subsequently, graft unions and rootstock bases of each plant were removed, triple-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. These sections were aseptically dissected to expose the xylem tissue. Four small (1×0.5 mm) sections were cut from the xylem tissue 1–2 mm adjacent to the graft union and also 1 cm from the rootstock base and plated on potato dextrose agar medium (PDA; Biolab, Johannesburg, South Africa) amended with 250 mg l^{-1} chloramphenicol. Isolation plates were incubated for 30 days at 23 °C under a diurnal light schedule (12 h light, 12 h dark) before cultures were microscopically identified. Infection levels of *Trichoderma* spp. and grapevine pathogens, i.e. *Phaeomoniella* and *Phaeoacremonium* spp. (Petri disease), *Cylindrocarpon* and *Campylocarpon* spp. (black foot), *Botryosphaeria* spp. (black dead arm) and *Phomopsis* spp. (dead arm), in the graft unions and rootstock bases were calculated as a percentage of the four dissected wood sections isolated from each part.

Statistical analyses

Certifiable plant yield percentages, root and shoot mass, and percentage incidence of *Phaeomoniella* and *Phaeoacremonium*, *Cylindrocarpon* + *Campylocarpon*, *Botryosphaeria* and *Phomopsis* spp., total pathogen (i.e. total of the aforementioned pathogens) and *Trichoderma* infection data were subjected to analyses of variance using SAS version 8.1 (SAS Institute, Cary, North Carolina USA). Student's *t*-Least Significant Difference was calculated at the 5% significance level to compare treatment means. A Pearson's correlation procedure was done to compare root and shoot mass means with certifiable plant yield percentages.

Results

Growth parameters

Significant year × treatment × grafting regime interaction was observed for the certifiable plant yield percentages ($P = 0.0014$; ANOVA table not shown). This was largely attributed to the Bronocide treatment, which yielded significantly less certifiable plants in both seasons and for both grafting regimes, and with percentages varying between 0.0% and 41.0% (Table 1). The three-factor interaction can also be attributed to statistically lower certifiable plant yields for all the machine-grafted treatments (19.2–48.8%) during the 2003/2004 season, except for the captan treatment (62.6%). Apart from this difference, none of

Table 1. Mean percentages of certifiable plant yield of machine and hand-grafted nursery grapevines of which the rootstock, scion and graftlings had been soaked in chemical or biological sanitation products during the 2002/2003 and 2003/2004 seasons

Treatment	Grafting regime	
	Machine-grafted	Hand-grafted
2002/2003 season		
Benomyl	67.4abcdefg	74.5abcd
Bio-sterilizer	58.9ghi	75.1ab
Bronocide	0.0n	38.3l
Chinosol	72.3abcdef	61.6fgh
Captan	62.6cdefgh	71.7abcdef
Sporekill	78.8a	74.9abc
Trichoflow-T	52.1hijk	68.6abcdefg
Water	71.4abcdef	74.1abcde
2003/2004 season		
Benomyl	43.0kl	69.3abcdefg
Bio-sterilizer	42.9kl	58.4ghij
Bronocide	19.2 m	41.0 kl
Chinosol	48.8ijkl	66.4bcdefg
Captan	62.6defgh	57.6ghij
Sporekill	46.5jkl	61.8efgh
Trichoflow-T	40.1kl	67.0abcdefg
Water	46.6ijkl	65.2bcdefg
LSD[a]	12.33	

[a]Least significant difference: means followed by the same letter do not differ significantly ($P < 0.05$).

the soak treatments performed better than the water controls in each season and grafting regime. However, the Bio-sterilizer (58.9%) and Trichoflow-T (52.1%) treatments yielded significantly less certifiable plants than the water control (71.4%) for the 2002/2003 machine-grafted regime. In 2002/2003, Chinosol (61.6%) also yielded significantly less certifiable hand-grafted plants than the water control (74.1%).

For root and shoot mass data, significant year × grafting regime interaction ($P = 0.0014$ and $P < 0.0001$, respectively; ANOVA tables not shown) was observed and significant grafting regime × treatment interaction ($P = 0.0337$) was observed for the root mass data. This was largely attributed to the same factors that affected the percentage certifiable plant yield data as these parameters showed a significant negative correlation with certifiable plant yield percentages ($P < 0.0001$), which was most likely due to reduced competition between plants as a result of reduced plant density. Root and shoot mass data are therefore not shown.

Infection levels

On average, total pathogen incidence in basal ends of rootstocks (30.0%) of uprooted nursery plants was substantially higher than that recorded for graft unions (13.5%). *Phaeomoniella* was the most commonly isolated pathogen from the water-treated control plants and at substantially higher levels from basal ends (24.6%) compared with graft unions (8.3%). The other trunk disease pathogens were isolated at markedly lower incidences from graft unions and basal ends of water-treated plants: *Phaeoacremonium* (3.6% from each), *Cylindrocarpon* + *Campylocarpon* (0.2% and 1.5%, respectively), *Botryosphaeria* (1.2% and 0.2%, respectively) and *Phomopsis* spp. (0.2% and 0.0%, respectively). *Trichoderma* spp. also occurred at low levels (0.1% and 0.2%, respectively).

Table 2. Analyses of variance for effects of season (Year), grafting regime and soak treatments on the percentages incidence of total pathogen (Pathogen), *Phaeomoniella* (Pch), *Phaeoacremonium* (Phaeo), *Cylindrocarpon* + *Campylocarpon* (Cyl), *Botryosphaeria* (Bot), *Phomopsis* (Phom) and *Trichoderma* (Tricho) spp. isolated from graft unions and basal ends of machine and hand-grafted nursery grapevines of which the rootstock, scion and graftlings had been soaked in chemical or biological sanitation products during the 2002/2003 and 2003/2004 seasons

Source		Pathogen		Pch		Phaeo		Cyl		Bot		Phom		Tricho	
	DF	MS	SL	MS	SL	MS	SL	MS	SL	MS	SL	MS	SL	MS	SL
Graft union															
Model	36	104.03	< 0.0001	64.08	< 0.0001	11.81	0.0105	0.74	0.0280	2.76	0.4979	0.16	0.3765	11.32	< 0.0001
Year	1	1.12	0.8704	0.18	0.9366	2.45	0.5897	2.00	0.0259	9.15	0.0581	0.34	0.1480	3.72	0.3231
Error A	6	38.66	0.1320	26.34	0.1362	7.54	0.3227	0.23	0.7903	1.68	0.7283	0.13	0.5217	3.21	0.5062
Graft regime (G)	1	448.57	< 0.0001	172.07	0.0014	59.21	0.0031	5.06	0.0011	2.25	0.3714	0.15	0.3169	6.31	0.1895
Treatment (Trt)	7	233.29	< 0.0001	162.38	< 0.0001	10.95	0.1150	1.20	0.0139	4.25	0.1695	0.13	0.5121	29.13	< 0.0001
G × Trt	7	86.44	0.0012	73.45	0.0002	14.67	0.0336	0.12	0.9606	1.67	0.7540	0.15	0.4106	14.07	0.0010
Year × G	1	57.44	0.1162	48.40	0.0828	2.78	0.5106	0.28	0.4304	0.01	0.9563	0.37	0.1116	0.42	0.7352
Year × Trt	7	89.22	0.0009	30.19	0.0756	12.09	0.0795	1.09	0.0233	4.62	0.1300	0.12	0.5762	6.24	0.1127
Year × G × Trt	6	23.89	0.4009	11.00	0.6503	8.56	0.2472	0.16	0.9017	0.68	0.9598	0.20	0.2221	5.31	0.1973
Error B	87	22.82		15.71		6.37		0.44		2.79		0.14		3.61	
Corrected total	123														
Basal end															
Model	36	342.50	< 0.0001	290.59	< 0.0001	11.95	0.0534	7.91	0.1995	0.38	0.2521	0.05	0.4950	9.24	< 0.0001
Year	1	386.77	0.0802	517.27	0.0017	55.10	0.1506	105.06	0.0012	0.00	0.9702	0.05	0.3632	18.21	0.1158
Error A	6	87.48	0.0818	17.89	0.8337	20.30	0.0223	3.15	0.8085	0.68	0.0588	0.05	0.4527	5.39	0.0532
Graft regime (G)	1	99.80	0.1395	75.89	0.1645	2.35	0.5839	15.56	0.1205	0.83	0.1119	0.05	0.3201	0.03	0.9180
Treatment (Trt)	7	1185.83	< 0.0001	1056.45	< 0.0001	20.41	0.0164	4.41	0.6749	0.29	0.5003	0.05	0.4641	23.22	< 0.0001
G × Trt	7	102.41	0.0350	106.74	0.0121	1.16	0.9937	6.74	0.3928	0.23	0.6630	0.05	0.4540	5.03	0.0607
Year × G	1	118.38	0.1080	46.39	0.2760	3.40	0.5096	5.00	0.3767	0.05	0.6822	0.06	0.2807	0.02	0.9214
Year × Trt	7	198.44	0.0003	107.86	0.0114	11.56	0.1815	6.29	0.4412	0.29	0.5146	0.05	0.4751	8.94	0.0019
Year × G × Trt	6	132.22	0.0115	136.17	0.0036	2.60	0.9164	3.04	0.8214	0.52	0.1484	0.06	0.3662	3.62	0.2016
Error B	87	44.88		38.61		7.76		6.33		0.32		0.05		2.48	
Corrected total	123														

The analyses of variance of the percentage incidences of total pathogen, *Phaeomoniella*, *Phaeoacremonium*, *Cylindrocarpon* + *Campylocarpon*, *Botryosphaeria* and *Phomopsis* spp., as well as *Trichoderma* spp. that were isolated from the graft unions and basal ends of rootstock are given in Table 2.

Graft unions

The statistically significant year × treatment interaction ($P = 0.0009$; Table 2) for total pathogen incidences in graft unions can be attributed to the inconsistent efficacy of Bio-sterilizer. During the 2002/2003 season, isolations from graft unions of Bio-sterilizer treated plants yielded markedly more pathogens (18.1%) than the water control (14.5%) and in the 2003/2004 season markedly less (7.8% vs. 12.5%; LSD = 4.89; data not shown). A year × treatment interaction was also observed for *Cylindrocarpon* + *Campylocarpon* incidence ($P = 0.0233$; Table 2), which was attributed to generally low incidences for all treatments (0.0–0.6%), except for the 2004 captan treatment (1.6%; data not shown).

For total pathogen incidences in graft unions, significant grafting regime × treatment ($P = 0.0012$; Table 2) interaction was also observed. None of the treatments differed significantly from the water control in machine-grafted plants (8.6%; Table 3), although markedly lower incidences were recorded for benomyl (3.9%). Significantly more pathogens were isolated from hand-grafted graft unions (water control = 18.4%) and Bronocide (3.2%), benomyl (4.8%), captan (7.8%), Sporekill (8.6%) and Chinosol (12.6%) treatments resulted in significantly lower incidences than the controls (Table 3).

Significant grafting regime × treatment interactions were also observed for *Phaeomoniella* ($P = 0.0002$) and *Phaeoacremonium* ($P = 0.0336$) incidences in graft unions (Table 2). *Phaeomoniella* was isolated at statistically higher incidences from hand-grafted graft unions (water control = 11.3%) compared with machine-grafted graft unions (water control = 5.3%; Table 3). In the latter graft unions, Bronocide (0.5%), Sporekill (2.1%), Bio-sterilizer (2.3%) and benomyl (2.5%) resulted in lower incidences than the controls, although only the Bronocide effect was significant ($P < 0.05$). In hand-grafted graft unions, however, *Phaeomoniella* incidence was significantly lower in Bronocide (0.6%), benomyl

Table 3. Mean percentage incidence of total pathogen (Path), *Phaeomoniella* (Pch), *Phaeoacremonium* (Phaeo), *Cylindrocarpon* + *Campylocarpon* (Cyl), *Botryosphaeria* (Bot), *Phomopsis* (Phom) and *Trichoderma* (Tricho) spp. isolated from graft unions of machine- and hand-grafted nursery grapevines of which the rootstock, scion and graftlings had been soaked in chemical or biological sanitation products

Treatment	Path	Pch	Phaeo	Cyl	Bot	Phom	Tricho
Machine-grafted							
Benomyl	3.9ab	2.5ab	1.3ab	0.0a	0.2a	0.0a	0.0a
Bio-sterilizer	8.0abcd	2.3ab	3.4bcdf	0.0a	1.7ab	0.5b	1.7ab
Bronocide	6.4abc	0.5a	5.3f	0.0a	0.6ab	0.0a	1.0ab
Chinosol	10.3cd	8.1def	1.9abc	0.0a	0.3a	0.0a	0.0a
Captan	7.5abc	3.9abc	0.8a	0.6abc	2.2b	0.0a	0.0a
Sporekill	6.1abc	2.1ab	2.2abcd	0.0a	1.8ab	0.0a	0.0a
Trichoflow-T	8.3bcd	5.1bcd	2.7abcd	0.0a	0.6ab	0.0a	6.4c
Water	8.6bcd	5.3bcd	1.9abc	0.0a	1.1ab	0.3ab	0.2ab
Hand-grafted							
Benomyl	4.8ab	1.7ab	1.6ab	0.8bc	0.6ab	0.0a	0.1ab
Bio-sterilizer	18.0f	11.9fg	4.5df	0.2ab	1.4ab	0.0a	0.8ab
Bronocide	3.2a	0.6a	1.6ab	0.2ab	0.9ab	0.0a	2.0b
Chinosol	12.6de	7.3cde	4.7df	0.3ab	0.2a	0.0a	0.1ab
Captan	7.8abcd	2.0ab	3.4bcdf	1.2c	1.3ab	0.1a	1.0ab
Sporekill	8.6bcd	3.5abc	4.3cdf	0.4ab	0.3a	0.1a	0.2ab
Trichoflow-T	16.9ef	12.2g	3.5bcdf	0.3ab	0.8ab	0.1a	1.5ab
Water	18.4f	11.3efg	5.3f	0.5ab	1.3ab	0.1a	0.0a
LSD[a]	4.89	4.06	2.59	0.68	1.71	0.39	1.95

[a]Least significant difference: means in each column followed by the same letter do not differ significantly ($P < 0.05$).

(1.7%), captan (2.0%) and Sporekill (3.5%) treated plants. Contrary to the case in machine-grafted plants, *Phaeomoniella* incidences in hand-grafted Bio-sterilizer treated plants (11.9%) did not differ from the control. *Phaeoacremonium* incidences did not differ between most treatments and the control (1.9%) in machine-grafted graft unions (Table 3), whereas Bronocide treatment resulted in significant higher incidences (5.3%). Compared with the control in machine-grafted graft unions, significantly higher *Phaeoacremonium* incidences were found in hand-grafted graft unions (water control = 5.3%). In this case, significantly lower incidences were observed for benomyl and Bronocide only (1.6% each).

Botryosphaeria and *Phomopsis* spp. were isolated at very low levels (0.2–1.8% and 0.0–0.5%, respectively; Table 3) and no significant treatment or grafting regime effect could be observed ($P > 0.05$; Table 2).

Significant grafting regime × treatment interaction was observed for *Trichoderma* ($P = 0.0010$) incidences in graft unions (Table 2). *Trichoderma* was isolated at significantly higher levels from machine-grafted graft unions of Trichoflow-T treated plants (6.4% vs. 0.2% of the water control), and only at slightly higher levels ($P > 0.05$) from hand-grafted graft unions (1.5% vs. 0.0% of the water control; Table 3). The saprophyte/biocontrol agent was also isolated at significantly higher levels from hand-grafted plants that were treated with Bronocide (2.0%).

Basal ends

Significant year × grafting regime × treatment interactions were observed for total pathogen ($P = 0.0115$) and *Phaeomoniella* incidences ($P = 0.0036$) in basal ends (Table 2). Mean incidences for these interactions are given in Table 4. Total pathogen and *Phaeomoniella* incidences in basal ends of water-treated machine-grafted plants did not differ significantly from those in hand-grafted plants in any of the seasons. However, the incidences in machine-grafted plants were significantly higher during the 2002/2003 season compared with the 2003/2004 season. These differences were also observed for hand-grafted plants, but were not significant. In the 2002/2003 season, all

Table 4. Mean percentages incidence of total pathogen (Path) and *Phaeomoniella* spp. (Pch) isolated from basal ends of machine and hand-grafted nursery grapevines of which the rootstock, scion and graftlings had been soaked in chemical or biological sanitation products during the 2002/2003 and 2003/2004 seasons

Treatment	Pathogen		*Phaeomoniella*	
	Machine-grafted	Hand-grafted	Machine-grafted	Hand-grafted
2002/2003 season				
Benomyl	5.9ab	6.3ab	3.8a	2.5a
Bio-sterilizer	22.5efgh	30.5hij	15.0bcd	24.7efgh
Bronocide	-	5.0ab	-	1.9a
Chinosol	20.0defg	24.4fgh	17.2de	18.4de
Captan	18.4defg	7.3abc	15.0bcd	4.7a
Sporekill	5.0ab	6.3ab	1.9a	4.4a
Trichoflow-T	20.6defg	34.2ij	15.0bcd	30.6gh
Water	39.1j	34.5ij	31.9h	28.0fgh
2003/2004 season				
Benomyl	2.5a	7.0abc	1.6a	1.3a
Bio-sterilizer	11.7abcd	23.2fgh	4.5a	17.7de
Bronocide	13.6bcde	8.9abc	7.1ab	3.0a
Chinosol	11.9abcd	13.1bcde	8.4abc	8.1abc
Captan	7.2abc	19.5defg	2.8a	14.1bcd
Sporekill	15.9cdef	12.0bcd	8.2abc	6.4ab
Trichoflow-T	23.4fgh	25.6ghi	21.1def	20.8def
Water	20.0defg	26.4ghi	16.6cde	22.0defg
LSD[a]	9.42	8.73		

[a]Least significant difference: means for Pathogen and *Phaeomoniella* followed by the same letter do not differ significantly ($P < 0.05$).

treatments resulted in significantly lower total pathogen and *Phaeomoniella* incidences in basal ends of machine-grafted plants (ranging 5.0% to 22.5% and 1.9% to 17.2%, respectively) compared with the water control (39.1% and 31.9%, respectively). This was also the case for most treatments in the hand-grafting regime in this season (ranging from 5.0% to 24.4% and 1.9% to 18.4%, respectively), apart from the Bio-sterilizer (30.5% and 24.7%, respectively) and Trichoflow-T (34.2% and 30.6%, respectively) treatments, which yielded statistically similar incidences to the water treatments (34.5% and 28.0%, respectively). In the machine-grafting regime, benomyl and Sporekill were consistently the best treatments, whereas benomyl, Sporekill, Bronocide and captan were the best treatments in the hand-grafting regime.

In the 2003/2004 season, total pathogen and *Phaeomoniella* incidences in the water-treated controls of machine (20.0% and 16.6%, respectively) and hand-grafted plants (26.4% and 22.0%, respectively) were lower and fewer treatments resulted in significantly lower incidences than the controls (Table 4). Total pathogen and *Phaeomoniella* incidences were consistently the lowest for the benomyl treatment for both grafting regimes (1.3–7.0%). In the machine-grafting regime, total pathogen and *Phaeomoniella* levels in basal ends of captan treated plants (7.2% and 2.8%, respectively), and *Phaeomoniella* incidences of Bronocide (7.1%) and Bio-sterilizer (4.5%) treated plants were also significantly lower than the respective water-treated controls. Likewise, in the hand-grafting regime, total pathogen and *Phaeomoniella* incidences of Bronocide (8.9% and 3.0%, respectively), Chinosol (13.1% and 8.1%, respectively) and Sporekill (12.0% and 6.4%, respectively) treated plants were also significantly lower than the controls. As was the case in the 2002/2003 season, Bio-sterilizer and Trichoflow-T yielded statistically similar incidences to the control treatments in the hand-grafting regime in 2003/2004. This was also the case for the Trichoflow-T treatment in the machine-grafting regime during this season.

No significant interactions were observed for *Phaeoacremonium* incidences and a statistically significant effect was observed for treatments only ($P = 0.0164$; Table 2). Benomyl (1.6%) and captan (0.7%) were the only treatments that differed significantly from the water-treated control (3.6%; data not shown).

For *Cylindrocarpon* + *Campylocarpon* incidence, significant effects were observed for year only ($P = 0.0012$; Table 2) as these pathogens were isolated at significantly higher incidences during the 2003/2004 season (2.8%) compared with the 2002/2003 season (1.0%; data not shown). However, no treatment ($P = 0.6749$) or grafting regime ($P = 0.1205$) effect could be observed (Table 2).

Botryosphaeria and *Phomopsis* spp. were isolated at very low levels from the basal ends of machine (0.21% and 0.04%, respectively) and hand-grafted (0.05% and 0.0%, respectively; data not shown). No significant effect for treatment ($P = 0.5003$ and $P = 0.4641$, respectively) or grafting regime ($P = 0.1119$ and $P = 0.3201$, respectively) could be observed (Table 2).

For *Trichoderma* incidence, significant year $\times$ treatment interaction ($P = 0.0019$) was observed (Table 2). Low levels of *Trichoderma* were isolated for most of the treatments in both seasons (0.0–0.5%; data not shown). However, statistically higher levels were recorded for Bronocide in both seasons (2.1% and 5.0%, respectively), and also for Bio-sterilizer (2.6%) during the 2002/2003 season. *Trichoderma* incidences were markedly higher in the basal ends of Trichoflow-T treated plants in the 2002/2003 season (1.3%), but not in the 2003/2004 season (0.2%).

Discussion

Soaking of propagation material is a standard practice in grapevine nurseries. However, most South African grapevine nurseries use the same untreated batch of water for 1–5 days, thereby inadvertently creating an unhygienic suspension of fungal and bacterial propagules from which propagation material might be infected during the soak period. Unfortunately such a suspension could not repeatably be used in this trial and clean tap water was used as control treatment. It should therefore be noted that the control treatment in this study depicts the best practice scenario, and not that of standard practice.

In this semi-commercial nursery trial, grapevine rootstock and scion cuttings were soaked in water (control), benomyl, Bio-sterilizer, Bronocide, Chinosol, captan, Sporekill and Trichoflow-T

during hydration (prior to cold storage) and prior to grafting (after cold storage), and the callused graftlings were dip-treated prior to planting in field nurseries. Natural infection levels in basal ends and graft unions of uprooted nursery grapevines were evaluated 8 months after planting. Total pathogen incidences in the water-treated control plants ranged from a 30% in basal ends to 13.5% in graft unions. *Phaeomoniella chlamydospora* was the most commonly isolated pathogen, followed by *Phaeoacremonium*, *Cylindrocarpon* + *Campylocarpon*, *Botryosphaeria* and *Phomopsis* spp. In corroboration with previous reports (Mugnai et al., 1999; Feliciano and Gubler, 2001; Rumbos and Rumbou, 2001; Phillips, 2002; Ridgway et al., 2002; Edwards et al., 2003; Halleen et al., 2003; Fourie and Halleen, 2002, 2004a), these results therefore clearly demonstrate that nursery grapevines are infected during the propagation stages and thereby act as a major dissemination source for these pathogens, especially for the Petri disease pathogens, *Phaeomoniella* and *Phaeoacremonium* spp. The infection levels observed in the uprooted dormant nursery plants might have been the result of infection of rootstock canes via vascular tissue from mother plants (Feliciano and Gubler, 2001; Ridgway et al., 2002; Edwards et al., 2003; Fourie and Halleen, 2002, 2004a), and/or wound infection from contaminated hydration or soak water, callus media, grafting equipment or nursery soils (Whiteman et al., 2002, 2003; Halleen et al., 2003; Damm and Fourie, 2005; Retief et al., 2005, 2006).

The growth parameters, certifiable plant yield and root and shoot mass, were not negatively influenced by repeated soak treatments in most suspensions, except for Bronocide (both seasons and grafting regimes), Trichoflow-T, Bio-sterilizer (2002/2003 machine-grafted plants) and Chinosol (2002/2003 hand-grafted plants). Differences in growth parameters between grafting regimes were furthermore observed during the 2003/2004 season only. The poor performance of machine-grafted plants during this season is unclear. It should, however, be noted that the machine-grafted plants were planted according to the planting practices normally followed for hand-grafted graftlings. As only one scion bud is machine-grafted to a rootstock, opposed to two buds in hand-grafting regimes, factors such as planting depth and soil moisture might have influenced the graftling's initial performance (pers. comm. Dirk Visser, KWV-Vititec, Paarl, South Africa).

Machine-grafted graft unions generally had lower pathogen incidences compared with hand-grafted graft unions. This can be directly attributed to the substantially smaller grafting wounds created in the machine-grafting regime. Furthermore, wounds created during hand-grafting frequently come into contact with unsterile hands of grafters from which it might be contaminated, especially if the grafters are handling unsterilised propagation material. In order to reduce the potential contamination of grafting wounds, it is therefore imperative that grafting personnel's hands and equipment be sterilised on a regular basis. Moreover, through the surface-sanitation of propagation material in soak treatments, especially prior to grafting, the amount of viable inocula on propagation material could be reduced, decreasing the chances of grafting wound contamination. The latter aspect was clearly demonstrated in this study.

Treatment of propagation material in a *T. harzianum* suspension (Trichoflow-T) did not affect the growth parameters of nursery grapevines negatively, except for lower certifiable plant yields in the 2002/2003 machine-grafting regime. *Trichoderma* incidences in graft unions were significantly higher in machine-grafted plants, but more so during the 2003/2004 season (data not shown), and reduced certifiable plant yields can therefore not be attributed to a detrimental effect of the *Trichoderma* infections in graft unions. The reason for the reduced certifiable plant yield is therefore unknown. Moreover, in similar experiments, a 1-h soak in Trichoflow-T prior to grafting did not adversely affect this parameter (Fourie and Halleen, 2004b). These authors furthermore reported that this treatment showed promise in a machine-grafting regime, but was not consistent in reducing the incidences of *Phaeomoniella* and *Phaeoacremonium* spp. Results from the present study confirm these findings as repeated Trichoflow-T soak treatments proved effective in reducing the levels of total pathogen and *Phaeomoniella* in basal ends of machine-grafted plants in the 2002/2003 season only. The increased efficacy of the biocontrol agent in the machine-grafting regime might be attributed to the hot callusing conditions being more suited to the environmental requirements of *T. harzianum*.

However, the reason for inconsistency between seasons is unclear. The potential use of this biocontrol agent as a wound protectant and growth stimulant in grapevine nurseries have been reported (Fourie et al., 2001; Fourie and Halleen, 2004b; Messina, 1999) and should be studied further to develop application methods that may ensure a more consistent efficacy.

In general, repeated soak treatments of propagation material in the tested chemicals resulted in reduced pathogen incidences in nursery grapevines. However, some products adversely affected growth parameters (as discussed earlier), and not all products yielded consistent results. In contrast with Bronocide's negative effect on certifiable plant yield, it proved to be one of the best sterilising treatments as it resulted in a reduction of pathogen incidence in basal ends and graft unions of hand and machine-grafted plants. It is possible that the concentration used in this study had a phytotoxic effect on propagation material and callused graftlings. This phytotoxic effect was most evident in the machine-grafting regime, which also resulted in increased *Phaeoacremonium* incidences in the graft unions. If this was the case, it might also explain the high occurrence of saprophytic *Trichoderma* spp. isolated from graft unions and basal ends of Bronocide-treated plants. Bio-sterilizer and Chinosol gave generally inconsistent results ranging from good to poor, depending on the specific pathogen, season or grafting regime. Benomyl, Sporekill and captan were consistently the best treatments as growth parameters were not negatively influenced by repeated soak treatments of propagation material in these solutions, and pathogen incidences in the uprooted plants were in most cases lower than that of the water-treated controls. Based on these results, and given the semi-commercial nature of this study, benomyl, captan or Sporekill can be recommended as soak treatments in grapevine nurseries.

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