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The effect of hot-water treatment on fungi occurring in apparently healthy grapevine cuttings

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Summary. Many grapevine nurseries are presently employing a hot-water treatment for propagation material as a prophylactic measure. The aim of this study was thus to determine the efficacy of this treatment in eliminating fungal endophytes from grapevine cuttings. The number of fungal colonies isolated from the hot-water treated cuttings 6 months after planting were not, however, significantly different (P=0.05) from that of the untreated cuttings. This was in contrast to where isolations were made directly after treatment, when no known living fungal pathogens were found inside treated vine tissue. These results therefore indicate that the hot-water treatment is effective in eliminating the most well-known fungal pathogens and endophytes from grapevine tissue. Further research now needs to be focused on combining this treatment with biological control agents in an attempt to delay recolonisation of tissue by fungal pathogens.

Key words: hot-water treatment, *Phaeomoniella*, young vine decline.

Introduction

Young vine decline, which is associated with various pathogens, is a very serious problem in most of the grapevine producing countries of the world (Whiting *et al*., 2001). Recent observations have suggested that fungi such as *Phaeomoniella chlamydospora* (W. Gams *et al*.) Crous & W. Gams and *Phaeoacremonium* species may play a dominant role in this decline (Crous *et al*., 1996, Crous and Gams, 2000), though experimental proof has been slow in confirming pathogenicity (Ferreira *et al*., 1999). Since researchers first became aware of these associations, however, there has been a frantic search for possible ways of eradication and control of the pathogens associated with young vine decline.

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Von Broembsen and Marais (1978) reported that subjecting vine cuttings to a hot-water treatment gave effective control of *Phytophthora cinnamomi*, without any visual phytotoxicity. Other researchers have also reported effective control of phylloxera, nematodes and Pierce's disease using this technique (Goheen *et al*., 1973). Using glass vials, spore suspensions and mycelial plugs, Whiting *et al*. (2001) found that a hot-water treatment may not be effective in reducing or eliminating *P. chlamydospora* and *Phaeoacremonium inflatipes* from vines. In contrast, however, this method is seen as effective against young vine decline by others (Waite, 1998). The aim of the present study, therefore, was to compare the fungal species occurring as endophytes within apparently healthy young vines and cuttings subjected to a 50°C hot-water treatment for 30 min, and in so doing determine the efficacy of this treatment for vine material.

Materials and methods

Hot-water treatment

Rootstock and scion vines (20–25 cm long, 1–1.5 cm diam.) were treated by dipping them in a hot-water bath at 50°C for 30 min, immediately followed by a cold-water bath for a further 30 min. For the first experiment 45 hot-water treated and 45 untreated grapevine cuttings (Shiraz 99B grafted onto AA219A [101-14]) that were externally disease free, were planted in a nursery in the Paarl region of the Western Cape province for a period of 6 months, after which they were uprooted and taken to the laboratory for isolations. For the second experiment, 100 shoots were cut from rootstocks (101-14) growing in a vineyard at Nietvoorbij, Stellenbosch in the Western Cape. The lower 20–25 cm of each shoot was taken as representative of one cutting. Fifty of these were subsequently submitted to a hot-water treatment.

Fungal isolation

Treated and untreated cuttings were stored in a cold room (10°C) for 7 days before being subjected to isolations. Roots from both experiments were surface sterilised using a three step sterilisation: 30 sec in 70% ethanol, 2 min in NaOCl (1%) and 15 sec in 70% ethanol. Ten root pieces were sampled per cutting. The cutting was then split open aseptically. Respectively 10 tissue pieces (2 mm×2 mm) were sampled from the inside of the crown and scion area of each cutting. Tissue pieces were plated on Petri dishes containing potato dextrose agar (PDA, Biolab, Midrand, Johannesburg, S.A.), amended with streptomycin (40 ppm), and incubated at 25°C.

Results and discussion

The primary aim of this study was to investigate the effect of a hot-water treatment on fungi occurring in grapevine cuttings. From Table 1 it is apparent that the number of fungal colonies isolated from the hot-water treated cuttings was not significantly reduced (P=0.05), compared to the untreated cuttings. The same was also true in the case of the more common vine pathogens occurring in the root, crown and scion areas of vine cuttings growing in the field for a period of 6 months.

No *Phaeomoniella* strains were obtained from the second experiment, and very few from the first experiment. The latter were all isolated from cuttings that underwent the hot-water treatment, suggesting that if the hot-water treatment had been effective, these cuttings were probably re-infected in the soil, or during the grafting process. No information is available about a possible soil-borne phase of *P. chlamydospora*, and this aspect is presently being investigated further. Of the other pathogens isolated *Fusarium* and *Cylindrocarpon* spp. proved to be dominant. Although the devastating effect of Cylindrocarpon black foot rot is well-known, not much is known about Fusarium wilt, and the importance of this disease complex will thus also have to be elucidated. Species of *Botryosphaeria* that cause black dead arm were also found, though in low numbers. Although it has recently been demonstrated that *Botryosphaeria* spp. can survive as endophytes in apparently healthy woody material (Mostert *et al*., 2000), not

Table 1. Endophytic fungi most frequently isolated from grapevine root, crown and scion areas 6 months after being planted out in the field. The figures indicate the total number of isolations from a given cutting region; e.g. 5/2 indicates 5 *Acremonium* isolates from roots of hot-water treated, and 2 isolates from untreated cuttings. Only those fungi that had an occurrence of more than 1% have been considered.

Fungal genera[b]	Treated/Untreated[a]		
	Root	Crown	Scion
Acremonium	5/2	1/1	19/6
Alternaria	7/11	17/10	45/35
Aspergillus	1/5	2/1	1/5
Botryosphaeria	3/3	14/3	9/19
Cylindrocarpon	5/49	27/54	3/5
Fusarium	85/48	43/27	106/139
Gliocladium	3/0	6/0	6/4
Macrophomina	0/8	0/0	–
Penicillium	30/7	20/8	6/3
Pestalotiopsis	1/4	9/8	18/22
Phaeomoniella	0/0	7/0	0/0
Rhizoctonia	0/0	0/3	0/9
Sterile white	2/10	5/6	8/6
Trichoderma	5/2	27/16	20/32

[a] Ten wood pieces were used for isolations per region from respectively 45 treated and untreated plants.

[b] Isolates with an occurrence of less than 1% include: *Balanium, Bipolaris, Chaetomium, Cladosporium, Curvularia, Epicoccum, Gonatobotryum, Myrothecium, Phoma, Phomopsis, Spadicoides, Ulocladium*.

much is known about the virulence and pathogenicity of these endophytic strains, and further research is now also being focused on these aspects. Other root pathogens that are common in soils in the Western Cape, namely *Macrophomina* and *Rhizoctonia* spp. were also recovered, though their importance to grapevine propagation remains to be determined.

In the case of the cuttings where isolations were made directly after treatment, the hot-water treatment as applied here completely eliminated fungi occurring in the stems of treated cuttings. These results therefore lead us to conclude that the hot-water treatment is effective in eliminating endophytes in the stems of grapevine cuttings, as well as most of the common pathogens. It is evident that the treated cuttings get re-infected in the field once planted out, and that the advantage of being "fungal free" may be short lived. This does, however, raise interesting possibilities of combining the hot-water treatment with a biological control agent such as *Trichoderma*, which could ensure that plants remain pathogen free for a longer period. Although the data obtained in the present study cannot adequately address all the questions pertaining to the young vine decline disease complex, it did indicate that the hot-water treatment was very effective against most of the pathogens associated with the decline of young vines.

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