

Slow Dieback of Grapevine in South Africa: Stress-Related Predisposition of Young Vines for Infection by *Phaeoacremonium chlamydosporum*

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***Phaeoacremonium chlamydosporum* causes slow dieback of nursery vines and young vines in vineyards. In a greenhouse trial it was observed that significantly more inoculated Chenin blanc vines grafted onto rootstock 101-14 Mgt, and subjected to water stress, exhibited dieback symptoms. More plants eventually died compared to inoculated vines not subjected to stress. Inoculation technique (stem inoculation vs soil inoculation) did not significantly affect disease incidence. Extensive plugging of the xylem tissue in inoculated plants was observed, eventually leading to slow dieback of these vines.**

Prior to the association of *Phialophora parasitica* Ajello *et al.* with dieback of grapevine in South Africa (Ferreira, Van Wyk & Venter, 1994), these symptoms, as well as decline of young vines, were attributed to poor propagation material, nematodes, unfavourable soil conditions, and fungal infections (Loubser & van Huyssteen, 1992). *Phialophora parasitica* is also associated with similar diseases in other woody plants and in some earlier reports the organism was identified as a *Cephalosporium* sp. (Hawksworth & Gibson, 1976; Hawksworth, Gibson & Gams, 1976). The taxonomic status of *P. parasitica* was subsequently re-evaluated and a new genus, *Phaeoacremonium*, was created to accommodate fungal isolates associated with wilt and decline diseases of woody plants (Gams *et al.*, 1996). From this study *Phaeoacremonium chlamydosporum* Crous *et al.* was the suggested binomial for the common grapevine dieback pathogen. However, it was suggested that more than one *Phaeoacremonium* species could be involved. The report by Scheck, Vasquez & Gubler (1998) that three species, i.e. *P. chlamydosporum*, *P. inflatipes* and *P. aleophilum* are involved in decline of young vines substantiates the suggestion of Crous *et al.* (1996). Occlusions in xylem tissue reported by Ferreira *et al.* (1994) were also associated with young vines showing symptoms of decline in California (D.W. Gubler, 1998, personal communication) and due to these black occlusions the disease is commonly known as "Black Goo" (L. Morton, 1997, personal communication) or *Phaeoacremonium* young vine decline (D.W. Gubler, 1998, personal communication).

Field observations on decline in young vines over the past five years led us to assume that increased susceptibility may result from a predisposing factor such as drought stress. The effect of drought stress on infection by *P. chlamydosporum* and disease development was therefore investigated.

MATERIALS AND METHODS

Isolation techniques: The *P. chlamydosporum* isolate used in the study was obtained from a Cabernet Sauvignon vine grafted onto rootstock 101-14 Mgt showing slow dieback symptoms. Small pieces of wood (0,5 x 2-3 mm long) were cut from the discoloured xylem tissue of the rootstock and placed on Potato Dextrose Agar containing Chloromycetin (250 mg/L). After 10 days of incubation at 25°C the fungus was transferred to Tea Leaf Agar (TLA) and incubated for 10 days at 25°C (Ferreira *et al.*, 1994), before subculturing for inoculation purposes on TLA before use.

Inoculation techniques: Inoculation experiments were conducted on one year old Chenin blanc vines grafted onto 101-14 Mgt and planted in sterile potting mixture (perlite : compost : pine bark : 1:1:1) in 2L plastic bags in a greenhouse. Two inoculation techniques were followed. Using the first technique, a small hole (8mm x 3 mm deep) was drilled into the rootstock near the soil surface. A mycelium plug, cut with a cork borer from a TLA culture of the fungus, was placed in the hole and sealed with a piece of cotton wool, impregnated with petroleum jelly. The plug was kept in position with masking tape wrapped around the plant. Control plants received only an agar plug from uninoculated TLA. When using the second technique, plants were removed from the pots and 20 mL of a macerated TLA culture of the fungus was spread onto the soil surface and thoroughly mixed with the top third of the potted soil. Plants were replanted after one third of the roots were pruned back. Control pots received 20 mL of uninoculated TLA, and treated as discussed above.

Water stress treatment: Half of the plants from each inoculation technique were subjected to drought stress in order to determine the effect of stress on disease development. Watering of

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these plants was withheld until the growing tip started to wilt, whereafter each pot received 200 mL of water. This watering regime was continued until plants were finally evaluated. Plants not subjected to stress were watered twice weekly. All plants were lifted nine months after inoculation for disease assessment.

Statistical procedures: A 2^3 factorial experiment was carried out in a randomised complete block design. The factors were two fungal treatments (with and without); two inoculation techniques (stem and soil) and two water stress treatments (with and without). These eight treatment combinations were randomly allocated within each of the three block replicates. Blocks were formed by position in the greenhouse and by stem diameter. An experimental unit consisted of 10 pots with one plant per pot.

The dieback symptoms were evaluated visually on a five point ordinate scale of 0-4 (0 = healthy; 1 = slight dieback symptoms; 2 = moderate dieback symptoms; 3 = severe dieback symptoms and 4 = dead).

The data were analysed using a general linear model technique. The PC-PI,1JM program originally written by Peter McCullagh was used to analyse the data. Comparisons of interest were tested by means of analyses of deviance (McCullagh & Nelder, 1989).

Classes 2, 3 and 4 were considered as fully diseased plants and the percentage diseased plants were calculated and subjected to analyses of variance, using procedure GLM of SAS V6:12 (SAS, 1996). Shapiro Wilk's test was performed to test for non-normality (Shapiro & Wilk, 1965).

RESULTS AND DISCUSSION

Significance was only evident for the main effects (Table 1). As expected, significantly more of the inoculated plants (stem as well as soil inoculation) fell in class 4 (dead plants) (Fig. 1 a), compared to the uninoculated control plants, which were more pronounced in class 0 (healthy plants). Extensive plugging of the

TABLE 1

Analysis of deviance on frequencies observed on a five-point ordinal scale for dieback of *Phaeoacremonium chlamydosporum* symptoms.

Source	df	MD	SL
Blocks	2	0,03	0,97
Fungus (F)	1	152,54	<0,01
Inoculation (I)	1	7,11	<0,01
Stress (S)	1	17,28	<0,01
F x I	1	2,59	0,09
F x S	1	1,69	0,16
I x S	1	0,11	0,70
F x I x S	1	0,03	0,84
Error	14	0,7581	

df = degrees freedom.

MD = Mean deviance.

SL = Significant level.

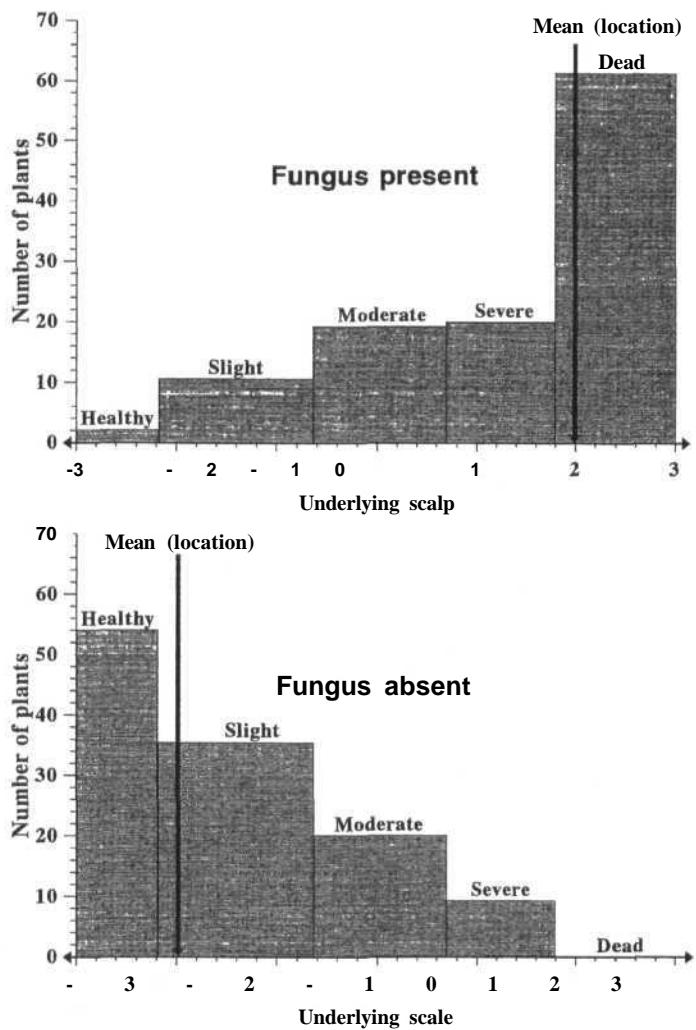


FIGURE 1a

Main effect of the fungus on the number of diseased plants in each disease class inoculated with *Phaeoacremonium chlamydosporum* and uninoculated plants (0 = healthy; 1 - slight dieback symptoms; 2 = moderate dieback symptoms; 3 = severe dieback symptoms and 4 = dead).

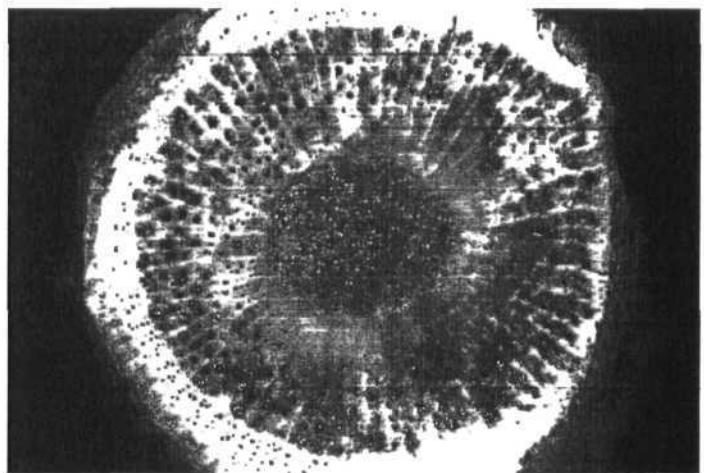


FIGURE 1b
Cross section through the stem of an affected vine.

xylem tissue of inoculated plants was evident when a cross cut was made through the stems (Fig. 1 b). In some of the stem-inoculated plants about 80% of the xylem tissue was plugged, while some of the xylem vessels showed discolouration resulting from a yellow-brown gumlike substance. Similar occlusions and yellow-brown gumlike material were found by Deacon & Scott (1983) in maize roots infected with *Phialophora zeicola*. These occlusions were primarily composed of pseudoparenchyma which completely filled the cortical cells of the host. A scanning electronmicroscopic study of the occlusions in grapevine xylem vessels of stems indicated that these occlusions were parenchymatous of origin (Ferreira *et al.*, 1994).

Although a significant difference was evident for inoculation technique (Table 1), stem-inoculated and soil inoculated plants fell in the same disease class (Fig. 1a) indicating that inoculation technique did not have a great influence. Slightly more of the stem-inoculated plants occurred in class 4 (dead plants) than those subjected to soil inoculation (Fig. 2). This trend was appar-

ent despite the fact that the vigour of soil inoculated plants was also disrupted because of root pruning.

Water stress significantly increased the number of diseased plants (Table 1). Significantly more of the plants subjected to water stress occurred in class 4 (dead plants) compared to plants which were not subjected to water stress (Fig. 3). The majority of samples from commercial vines suffering from dieback and analysed at ARC-Nietvoorbij, suffered from drought (Ferreira, unpublished). Deacon & Scott (1983) suggested that *P. zeicola* is a weak pathogen which causes severe disease symptoms only when the plant is predisposed by damage. From the present study it is evident that water stress is a predisposing factor for *P. chlamydosporum* infection and the subsequent slow dieback of grapevine. In California the predisposing factors in vineyards relate to chilling injury and/or irrigation problems (D.W. Gubler, 1998, personal communication).

The occlusions formed in the xylem tissue will reduce the effi-

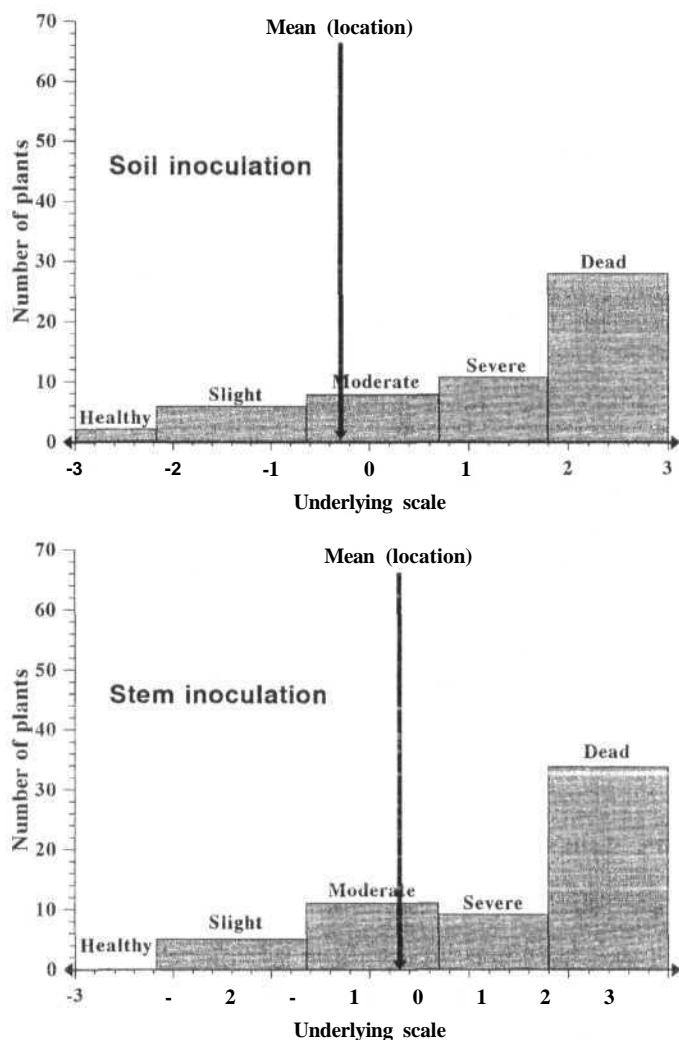


FIGURE 2

Main effect of the inoculation method (soil and stem inoculation) on the number of diseased plants in each class (0 = healthy; 1 = slight dieback symptoms; 2 = moderate dieback symptoms; 3 = severe dieback symptoms and 4 = dead).

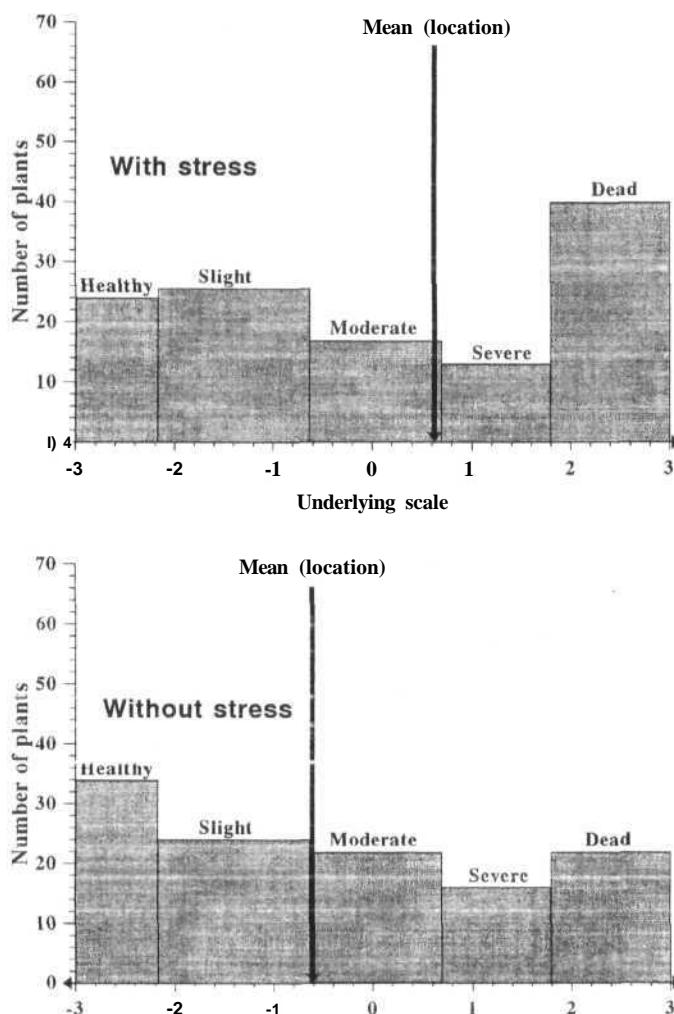


FIGURE 3

Main effect of water stress on the number of plants in each disease class (0 = healthy; 1 = slight dieback symptoms; 2 = moderate dieback symptoms; 3 = severe dieback symptoms and 4 = dead) after inoculation with *Phaeoacremonium chlamydosporum*.

ciency of water and mineral nutrient uptake, resulting in infected plants being unable to handle conditions where healthy plants normally are unaffected. Apart from reduction in water uptake, early infections of rootstocks in nurseries may also deplete nutrient reserves in young vines. This reduced vigour results in mortality soon after plants are planted in a vineyard.

The pathogen is usually restricted to the rootstock until one year after grafting (Ferreira, unpublished), whereafter it spreads to the scion parts. Should the pathogen be able to exist endophytically in rootstock, spread of disease from nursery material and mother blocks to new vineyards is obvious. This aspect should urgently be clarified and the necessary precautions implemented.

The results indicate that water stress is a predisposing factor for infection of nursery and young vines by *P. chlamydosporum*. Abolishing of stress factors in nurseries and vineyards could therefore minimise the occurrence of the disease.

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