Fungi Associated with Dieback and Pruning Wounds of Grapevines in South Africa

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Isolations were made from discoloured tissue of grapevines showing dieback symptoms in the winter rainfall region. Fifteen fungal species were isolated, of which *Sphaeropsis* sp. *Fusarium oxysporum, Eutypa lata* (anamorph: *Libertella blepharis* A.L. Smith), *Pestalotia quepini* and *Botrytis cinerea* could be designated as parasites. *E. lata* was the most probable cause of the dieback phenomenon. Additional isolates representing ten fungal genera were isolated from discoloured wood in spurs from visually healthy grapevines while organisms representing 14 genera were isolated from lesions in grapevines exhibiting dieback symptoms. *Aspergillus* sp. was more common in the healthy and marginal zones while *Sphaeropsis* sp. occurred more often in the healthy zone and *Phylosticta* sp. in the discoloured zone of pruning stubs. In lesions from vines with dieback symptoms, *Alternaria alternata* was isolated more regularly from the marginal zone between healthy and discoloured wood, while *E. lata* and *Trichoderma harzianum* were isolated more often from the older part of the lesion. A succession of fungi in the colonisation of pruning wounds and in dieback lesions is suggested.

Dieback of grapevine, caused by *Eutypa lata* (Pers: Fr.) Tul. syn. *Eutypa armeniacae* Hansf & Carter (Anamorph: *Libertella blepharis* A.L. Smith) occurs worldwide (Petzoldt, Moller & Sall, 1982). This disease is responsible for considerable loss in yield (Ferreira, 1988) and is one of the main causes of a shortened production life of vineyards (Moller & Lehoczky, 1980). Ferreira (1988) found another Ascomycete, *Cryptovalsa* cf. *ampelina* to be abundant on one-year-old grape prunings and older dead wood. The anamorph stages of these two fungi cannot be distinguished morphologically. It is therefore possible that isolations from dieback vines can yield both fungi. In this paper however, isolates from dieback vines, which coincide morphologically with *Libertella blepharis,* were designated *Eutypa lata.*

According to Bolay & Moller (1977), dieback of grapevines does not occur in dry areas and the rainfall must be higher than 300 mm for the formation of perithecia of the fungus on dead wood. The mean annual rainfall in the Western Cape Province ranges from 286 mm at Worcester to 1073 mm at Constantia.

Isolations from grapevines and apricot with dieback symptoms almost always yield pure cultures of *E. lata* (Bolay & Moller, 1977; English & Davis, 1978; Moller & Kasimatis, 1978; Moller & Lehoczky, 1980; Petzoldt, Moller & Sall, 1981; Glawe, Dilley & Moller, 1983). Some scientists, however, isolated additional fungi from dieback infected grapevines (Chiarappa, 1959; Marais, 1974; English & Davis, 1978; Bisiach & Minervini, 1985).

Pruning wounds in vines lead to discolouration of the underlying wood and are also the entry points for micro-organisms. Evidence suggests that micro-organisms (bacteria and fungi) other than wood decay fungi also play a role in the discolouration of wood in trees (Shortle, 1979), while Blanchette & Shaw (1978) claim that decay fungi can occur after or together with other micro-organisms. According to Shibo (1967, 1972), Shortle, Tatter & Rich (1971) and Shortle & Cowling (1978) a succession of micro-organisms occurs after wounding of live trees, in which decay fungi follow bacteria and imperfect fungi which act as pioneer colonists.

The purpose of this study was to determine which fungi are associated with dieback and to establish whether different fungi were associated with different zones of discolouration in dieback lesions and in wounds initiated by pruning.

MATERIALS AND METHODS

Isolation of fungi from dieback vines in different localities

Samples of grapevines with dieback symptoms were collected from 22 different localities throughout the winter rainfall region as indicated in Fig. 1. Four vines per locality

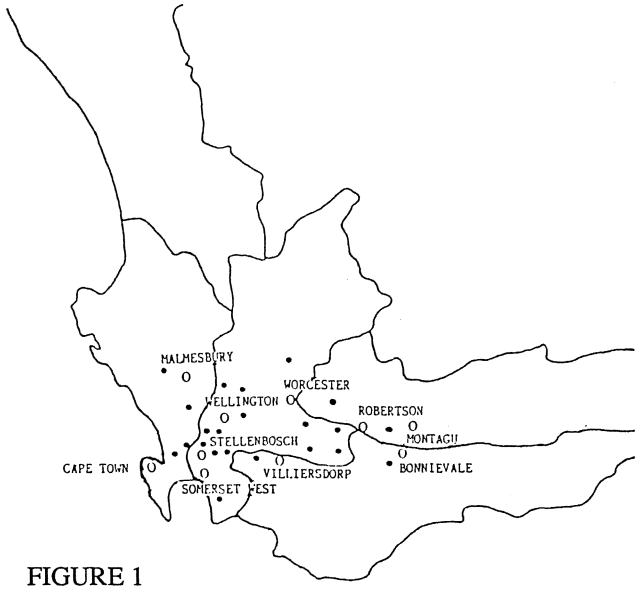

FIGURE 1
Localities where dieback vines were collected.

showing typical dieback symptoms were cut off approximately 200 mm above soil level and sawn longitudinally to expose the affected wood. Forty wood chips of 5 x 5 x 3 mm (ten per vine) were taken from the dead wood, surface sterilised in 0,5% sodium hypochlorite and then placed on potato dextrose agar (PDA) in petri dishes. Initial isolations on PDA, malt agar and carrot agar indicated no difference in the fungal species isolated on these three media. Petri dish cultures were incubated under a combination of fluorescent and black light at 25°C with a 12h photo period. After 5 days of incubation fungi were examined microscopically and tentatively identified on morphological characteristics. All fungi that did not sporulate were transferred to fresh medium and incubated as before. The formation of stylospores (anamorphal stage) were used to identify *E. lata*/*C.* cf *ampelina* (Ferreira, 1988). All species, except *Acremonium* sp and *Phylosticta* sp which could not be successfully recultured after storage on PDA in McCartney bottles, were subsequently sent to the Mycology unit, Plant Protection Research Institute, Pretoria, for confirmation of identity.

Isolation of fungi from one year old cane spurs

Twenty five-millimeters long wood samples (four replications of ten each) from spurs, each containing a one-year-old pruning wound, were collected randomly during the dormancy stage from a one hectare fourteen-year-old Cape Riesling (Crouchen blanc) vineyard at the VORI, Stellenbosch. Each spur was split longitudinally into quarters to expose discoloured and healthy wood. Three zones were distinguished visually: (i) discoloured, (ii) healthy and (iii) marginal wood (between healthy and discoloured wood). A wood chip (4 x 4 x 3 mm) was cut from each of these zones allowing a border of not less than 2 mm between adjacent zones.

Following surface sterilisation in sodium hypochlorite containing 0,5% available chlorine, the four wood chips from each zone per spur were placed on malt extract agar (MA), containing choloromycetin (500 mg/l), in a petri dish.

After an incubation period of 7 days at 24°C, fungal growth from each wood chip was examined microscopically and the fungi identified. Significant differences in the occurrence of fungi in the different zones were determined with Friedman's two-way analysis of variance by rank sums (Siegel, 1956).

Isolations of fungi from vines showing dieback symptoms

In the same vineyard as above vines with dieback symptoms were cut off approximately 100 mm above soil level. All shoots were removed and the vines split longitudinally with a bandsaw to expose discoloured wood. Four billets 3 mm thick (two from each half) were cut lengthwise. From the exposed surfaces, wood chips (5 x 5 x 3 mm) were cut from three visually distinct zones: (i) the margin between healthy and discoloured wood of the extending lesion, (ii) the middle of the lesion, and (iii) from the back of the lesion (wound where infection originated). Wood chips were surface sterilised as described for pruning stubs. Four chips from each zone per billet were placed on MA in the same petri dish. One hundred and twenty-eight lesions, in some cases more than one lesion from the same vine, were sampled.

After incubation for 7 days at 24°C, fungal growth was examined microscopically and the fungi identified. Significant differences in the occurrence of fungi in the different zones were determined as for cane spurs. Fungal species were stored on potato dextrose agar in McCartney bottles and those that could be successfully recultured at the end of the survey, were sent to the Mycology unit, Plant Protection Research Institute, Pretoria, to confirm original identification.

RESULTS AND DISCUSSION

Isolations of fungi from dieback vines in different localities

Eutypa lata was absent in only three of the localities sampled (Table 1). According to Petzoldt *et al.* (1981), 25 wood chips per vine are needed to isolate *E. lata* successfully from the border zone between healthy and discoloured wood, and if one of the wood pieces yielded *E. lata,* such a vine was regarded as being infected by the fungus. It is possible that *E. lata* would have been isolated from all the localities if a larger number of wood pieces had been used for isolation. Fifteen fungal species were isolated from dieback vines (Table 1).

Amongst the isolated fungi, *Sphaeropsis sp, E. lata, Fusarium oxysporum, Phomopsis viticola, Botrytis cinerea* and *Pestalotia quepini,* can be designated as parasitic fungi. The remaining fungi are assumed to be saprophytes. The occurrence of *F. oxysporum, P. viticola, B. cinerea* and *P. quepini* however, was too low to be considered as the cause of dieback. Although the *Sphaeropsis* sp. can also be considered parasitic, the ability of *E. lata* to form a toxin, which causes *in vitro* wilting of grapevine leaves and plantlets (Mauro *et al.* 1988), makes it the most probable pathogen for the dieback disease phenomenon.

Results in Table 1 further show that the occurrence of species differed between localities. This difference could be attributed to climatic factors or the age of the vines sampled. The general occurrence of *Chenin blanc* in South Africa may be the reason for the high number of dieback samples collected from this cultivar.

Isolations of fungi from one-year-old spurs

Ten fungal species were isolated from one-year-old spurs and all occurred in each of the three zones (Table 2). Population differences between the three zones were only significant in the case of three of the fungi isolated, namely *Aspergillus* sp., *Phylosticta* sp. and *Sphaeropsis* sp.

The significant higher occurrence of *Aspergillus* sp. in the healthy and marginal zones compared to the discoloured zone, and *Sphaeropsis* sp. in the healthy zone, indicated that these two species possibly act as pioneer organisms in the colonisation of pruning wounds. The concept of so-called "pioneer" organisms in the colonisation of wounds in trees is well documented (Shigo, 1967, 1972; Shigo & Hillis, 1973; Shortle & Cowling, 1978). Although only three fungal species were found to be significantly more common in any zone, the results indicate a possible succession of fungi in discolouration and decay of wood following wounding. In this regard it was established that *Aspergillus* sp. and *Sphaeropsis* sp. were more common at the margin of the extending lesion while *Phylosticta* sp. was more common in the older part of the wound. This supports the results of Etheridge (1961), Good & Nelson (1962), Shigo (1967, 1972), Tattar, Shortle & Thich (1971) on succession of organisms in decay of wood after wounding. According to Shigo & Hillis (1973) micro-

TABLE 1.
Occurrence of fungi in dieback affected grapevines from different areas in the Western Cape.

Cultivar/ Locality	Penicillium purpurascens	Acremonium sp	Sphaeropsis sp	Eutypa lata	Alternaria allernata	Aspergillus sp	Fusarium oxysporum	Epicoccum purpurascens	Phomopsis viticola	Trichoderma harzianum	Phyfostica	Gonatobotrys sp	Botrytis cinerea	Pestalotia guepini	Dactylosporium sp	Total spp
Chenin blanc Stellenbosch/Lynedoch	28	36	6	5	1	1	1	1		1		2		1	1	12
Chenin blanc Stellenbosch/Somerset West	39	3	4	3	3	2	1	2		2	1			1		11
Chenin blanc Malmesbury	23	39	7	1	19	6	3	10					1	1		10
Chenin blanc Stellenbosch/Durbanville	15	3	1		5	1	7	1		1		6				9
Chenin blanc Robertson	34	27	13	3	11	1		2		1			1			9
Chenin blanc Wellington	14	18	8	2	9	21		3					6			8
Chenin blanc Robertson	6	10	3	3	4	3		4		3						8
Chenin blanc Stellenbosch/Lynedoch	4	9	2	27		4			9			1				7
Chenin blanc Cape Town/Constantia	37	5		8	2	2		1		7						7
Chenin blanc Villiersdorp	4	5	6	4	4	2			9							7
Chenin blanc Wellington	7	18	8		6	4		2								6
Chenin blanc Stellenbosch/V O R I		14		2	4	1		2							1	6
Chenin blanc Malmesbury			12	2	5	4		2								5
Chenin blanc Worcester	19	27	16			29										4
Cabernet Sauvignon Stellenbosch/V O R I	5	1	11	25	2	3		3		2				2	1	10
Cabernet Sauvingon Stellenbosch/Klapmuts	8	8	30	10	13	3	1	7		1						9
Cabernet Sauvignon Stellenbosch/Bottelary	1	9		5	16	6	4	3		2				1		9
Cabernet Sauvignon Stellenbosch/Lynedoch	3	5		21	2	5					17				1	7
Riesling Stellenbosch/VORI	23	20	7	2	8	2	3	6			1					8
Riesling Stellenbosch/Elsenburg	4	17	10	5	3				10							6
Muscat d'Alexandrie Montagu	37	24	22	1	2	3	1							1		8
Pinotage Stellenbosch/Klapmuts		3		4	2	5	2									5
Total isolates	310	298	166	133	121	118	51	49	28	20	19	9	6	6	5	

Figures represent number of isolates out of a possible total of 40.

TABLE 2
Occurrence of fungi in three zones in one-year-old grapevine spurs.

Zone	FUNGAL SPECIES									
	Aspergillus sp	Phylosticta sp	Sphaeropsis sp	Alternaria alternata	Eppicoccum purpurascens	Phomopsis Viticola	Candida sp	Pestalotia guepini	Curvularia sp	Stemphylium sp
Healthy	96,5a	66,5a	92,0a	70,5a	69,5a	82,5a	85,0a	84,0a	84,0a	80,0a
Marginal	88,5a	78,5ab	78,0ab	80,0a	87,0a	87,0a	77,5a	79,0a	79,0a	79,5a
Discoulored	55,0b	95,0b	70,0b	89,5a	83,5a	71,5a	77,5a	77,0a	77,0a	80,0a

Numbers represent rank sums. Numbers in columns followed by different letters differ significantly ($p \leq 0,05$)

organisms that inhabit wood in living trees have the greatest survival advantage when they infect wounds in a sequential manner by which pioneers alter the substrate to the advantage of the other micro-organisms that follow.

Isolations of fungi from vines showing dieback symptoms

Isolates representing fourteen fungal species were obtained from lesions in dieback vines (Table 3). All species were present in each of the visually distinct zones. Of these species, only three were significantly more prevalent in one of the three zones.

TABLE 3
Occurrence of fungi in three zones from lesions in grape vines showing dieback symptoms

Zone	FUNGAL SPECIES													
	Penicillium purpurascens	Candida sp	Alternaria alternata	Eppicoccum purpurascens	Pestalotia guepini	Eutypa lata	Sphaeropsis sp	Fusarium oxysporum	Trichoderma harzianum	Cephalosporium sp	Aspergillus sp	Gonatobotrys sp	Botrytis cinerea	Phomopsis viticola
Marginal zone	200,5a	193,0a	217,0a	202,0a	192,5a	181,0a	192,5a	186,5a	188,0ab	194,5a	195,0a	192,0a	198,5a	192,0a
Middle	193,0a	193,0a	187,0b	191,5a	197,0a	204,5ab	185,0a	192,5a	180,5a	187,0a	190,5a	192,0a	191,0a	195,0a
Back	184,0a	191,5a	173,5b	184,0a	188,0a	212,0b	200,0a	198,5a	209,0b	195,0a	192,0a	193,5a	188,0a	190,5a

Numbers represent rank sums
Numbers in columns followed by different letters differ significantly ($p \leq 0,05$)

The significant occurrence of *A. alternata* sp. in the marginal zone between healthy and discoloured wood, while *E. lata* sp. and *T. harzianum* sp. were more prevelant in the oldest part of lesions in dieback vines, serves to demonstrate further a possible succession of fungi in the colonisation of vine pruning wounds. *Eutypa lata* and *T. harzianum,* however, never occurred together in the same lesion, possibly because of the antagonistic properties of the latter fungus (Dennis & Webster, 1971a; 1971b). Several workers found that wound susceptibility to *E. lata* decreased with increasing wound age (Carter & Moller, 1970; Carter & Price, 1975; Ramos, Moller & English, 1975; Moller & Kasimatis, 1980; Petzoldt *et al.*, 1981, 1982). This decrease in wound susceptibility is ascribed to rapid wound colonisation by other micro-organisms (Carter & Moller, 1970), which inhibit the growth of *E. lata* due to a possible competition for nutrients. Petzoldt *et al* (1981), however, ascribe this decrease in wound susceptibility to natural wound healing. From the present study it appears that fungi may colonise grapevine wood in a successive manner and it is possible that some of the fast growing early colonisers can inhibit the growth of *E. lata* as suggested by Carter & Moller (1970). Another possibility is that fresh wounds cannot readily be invaded by *E. lata* and that the early invaders predispose the wood for later growth by *E. lata* and other wood invaders. This succession of micro-organisms will be ever changing depending on age of the wound, nutritional status of the wood and environmental factors.

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