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Investigation into the presence of fungi associated with esca of young vines

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Summary. Thirty one 5 to 6-year-old grapevines with esca foliar symptoms, aspecific foliar symptoms, apoplexy and no symptoms at all were uprooted and examined in the laboratory for type of wood deterioration and presence of fungi. Various types of deterioration were recorded in the wood, three of which were found in all vines: necrosis originating in the cane stumps, sparse blackish streaks or blackish halos around the rootstock pith. Spongy decay was observed on six of the eight grapevines with the foliar symptoms of esca. Blackening of some roots was found on apoplectic vines. Among the fungi involved in esca, *Phaeoacremonium chlamydosporum* was isolated especially from the blackish halos around the rootstock pith, while *Eutypa lata* only from cane stump necrosis; but both were present on grapevines with and without foliar symptoms. *Fomitiporia* sp. was present only in spongy decay on vines with foliar esca symptoms. This fungus seems able to produce spongy decay only in necrotic tissues, but the necrosis itself could also be caused by non-parasitic factors. It was difficult to relate foliar symptoms to pathogen presence; it is necessary to examine a far larger number of samples to gather more accurate information.

Key words: esca, grapevine, internal and foliar symptoms, fungal colonization.

Introduction

The interest in vines infected with esca has led researchers to direct their studies towards young plants, in order to acquire a better understanding of the aetiology of this complex disease, which is not yet fully elucidated. Until the 1980s, two lignicolous basidiomycetes, *Phellinus igniarius* (L.:Fr.) Quélet and to a lesser extent *Stereum hirsutum* (Wild.) Fries, were considered the causal agents of esca. However, in 1959 Chiarappa isolated a *Cephalosporium* sp. which he supposed to have some role in the decay process. At the end of the 1980s, Larignon and Dubos (1987) suggested that a succession of fungi was involved in the disease process: *Eutypa lata* (Pers.:Fr.) Tul. & Tul. and *Cephalosporium* sp. were pioneer fungi, while a *Phellinus* sp. was a secondary invader. Soon after, *Phialophora parasitica* Ajello, Georg & Wang was also ranked among the pioneer fungi by Larignon (1991). Recently, the grapevine isolates of *Cephalosporium*, *Phialophora* and also *Acremonium* were included in the new genus *Phaeoacremonium* (Crous *et al*., 1996). Moreover, a more accurate analysis of *Phellinus* fruiting bodies showed that this fungus was actually *Fomitiporia* (*Phellinus*) *punctata* (Fr.) Murrill (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Cortesi *et al*., 2000).

In opposition to the hypothesis that *F. punctata* was only a secondary invader, Mugnai *et al*. (1996b) and Chiarappa (1997) reported that this fungus could also act as a primary pathogen, if a suitable wound permitting penetration and sufficent time were provided. In the light of this finding, it was

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supposed that esca could be an association of two distinct diseases with similar foliar symptoms: 1. brown wood-streaking or "young esca", caused by one or more species of *Phaeoacremonium*, and 2. white rot, caused by *F. punctata* (Mugnai *et al*. 1999). However, all these assumptions made by different Authors were not supported by complete fulfilment of Koch's postulates: artificial inoculations with fungi on vine trunk and branches did not reproduce the foliar symptoms of esca (Graniti *et al*., 1999).

In order to contribute to the knowledge of this complex puzzle, the Istituto Sperimentale per la Viticoltura of Conegliano began a series of studies on young vineyards, the first results of which are reported here.

Materials and methods

The two vineyards under study were established with different cultivars in spring 1992 and Sylvoz-trained with one-armed cordon and were located in different pedological areas of Treviso province. The vineyards were surveyed from 1993 and for each plant, any foliar symptoms indicative of wood diseases was recorded. Between 1997 and 1998, 31 vines were uprooted and examined in the laboratory; of these, 10 had never shown any foliar symptoms, 10 exibited aspecific symptoms, 8 had symptoms of esca, the remaining 3 were affected by apoplexy.

The uprooted vines were sectioned to identify deteriorated wood tissues, which were divided into 7 types (see Table 1). Chips of both deteriorated and healthy wood tissue were incubated at 25°C on malt extract agar, amended with tetracycline hydrochloride (50 mg/l), for isolation of fungi. The main fungi isolated were identified by comparing with strains obtained in a previous study (Serra, 1999).

Results

Some types of wood deterioration were found in all the 31 plants examined. These were: necrosis, usually involving the node alone and originating in the cane stumps (residues of old pruning) (A2); sparse blackish streaks (D) and a blackish halo around the rootstostock pith (A3).

In plants with foliar symptoms defined as aspecific (mainly chlorosis and necrosis of the edge and/or lamina of the leaf) and in asymptomatic plants, there was no deterioration other than those of the three types described above. The only exception was a plant with a few stunted shoots on the dry cordon where a brown sectorial area (A1) was noted.

Plants with the typical foliar symptoms of esca (interveinal islands of chlorotic and necrotic tissue sometimes joining to form a tiger-striped pattern) were first noticed in 1997 and 1998; symptom intensity was medium to low. Besides the types of wood deteriorations common to all plants examined, a pale yellow soft rot (spongy decay - B) was observed in 6 of the 8 plants with esca foliar symptoms. No correspondence was ever observed between symptomatic canes and the location of spongy decay or between the extent of such decay (which was always very restricted) and the severity of the foliar symptoms. Spongy decay developed only within pre-existing necrosis, usually cane stumps necrosis. Only in one vine did spongy decay extend for a short distance inside a sectorial necrosis. In all cases, spongy decay began from cracks and pruning cuts that allowed water stagnation; for example from a crack caused by faulty disbudding of suckers, where an old spur covered the cordon, or on the flat side of a S-shaped graft union.

In the plants affected with apoplexy there was evidence of blackening of some lateral roots. This blackening (A4) spread out beneath the bark to the rootstock and in a lesser degree to the trunk. On one of these vines too spongy decay was observed inside a necrosis originating in a large cane stump.

Of the 867 wood chips collected, most were taken from healthy tissue and from the deteriorated tissues of types A2 and A3 that were present in all the plants examined. Only 363 chips (i.e. 41.3% of the total) developed one or more fungal colonies amounting to a total of 400 colonies.

Most of the fungi isolated consisted of sterile mycelium (Table 1) that was mostly located in the sectorial necrosis (A1) and in necrotic tissue of type C (spongy decay and firm necrosis surrounding it). Of the fungi thought to be involved in esca, *Phaeoacremonium chlamydosporum* W. Gams *et al*. was isolated mostly from the blackish halo around the rootstock pith (A3), *E. lata* was found almost exclusively in type-A2 necrosis and *Fomitiporia* sp.

Table 1. Percentage of colonies of the most frequent fungi (>1% over the total amount, TOT) isolated from the different categories of tissues (A1 to H) [a].

Fungi	TOT	A1	A2	A3	A4	B	C	D	H
Acremonium alternatum	3[b]	0	5.4	1.5	0	0	0	5.7	1.3
Alternaria sp.	5.3	0	10.1	0	0	3.4	0	8.6	2.7
Cladosporium sp.	2.3	0	0	0	7.1	3.4	0	14.3	2.7
Coniothyrium sp.	4.8	0	1.4	0	7.1	6.9	10	8.6	12
Dendrophoma pleurospora f. *vitigena*	6.3	0	1.4	6.1	0	0	0	17.1	17.3
Fomitiporia sp.	4.3	0	0	0	0	44.8	20	0	0
Fusarium spp.	2.3	0	1.4	0	28.6	6.9	0	0	1.3
Lecythophora sp.	4	0	0.7	21.2	0	0	5	0	0
Eutypa lata	5.8	0	14.9	0	0	0	5	0	0
Mycelia sterilia	29	61.5	33.8	16.7	14.3	31	60	17.1	24
Penicillium spp.	1.3	0	0	1.5	0	0	0	0	5.3
Pestalotia sp.	1.3	0	0	1.5	0	0	0	0	5.3
Phaeoacremonium chlamydosporum	8.8	0	3.4	37.9	0	0	0	11.4	1.3
Phaeoacremonium sp.	1	0	2	0	0	0	0	0	1.3
Phomopsis sp.	4	15.4	7.4	4.5	0	0	0	0	0
Sphaeropsis sp.	6.3	23.1	11.5	0	21.4	0	0	5.7	0
Others	2	0	0.7	0	0	3.4	0	2.9	6.7
Undetermined	8.8	0	6.1	9.1	21.4	0	0	8.6	18.7

[a] A1 = brown sectorial necrosis; A2 = necrosis originating from cane stumps; A3 = blackish halo around the rootstock pith; A4 = underbark blackening; B = spongy decay; C = spongy decay and firm necrosis (A1 or A2) surronding it; D = blackish streaks; H = healthy tissue.

[b] Percentages were calculated on 400 total colonies, 13 colonies isolated from A1 woody chips, 148 from A2, 66 from A3, 14 from A4, 29 from B, 20 from C, 35 from D and 75 from H.

Table 2. Percentage of grapevine, showing or not showing foliar symptoms, which harboured each of the listed fungi.

Fungi	TOT	ASYM	ESCA	ASPE	APO
Acremonium alternatum	28.1[a]	20	25	40	33.3
Alternaria sp.	40.6	60	50	30	0
Cladosporium sp.	18.8	0	37.5	20	33.3
Coniothyrium sp.	28.1	10	50	40	0
Dendrophoma pleurospora f. *vitigena*	18.8	0	62.5	10	0
Fomitiporia sp.	21.9	0	75	0	33.3
Fusarium spp.	15.6	10	25	10	33.3
Lecythophora sp.	28.1	40	37.5	20	0
Eutypa lata	40.6	40	75	30	0
Mycelia sterilia	87.5	100	87.5	80	100
Penicillium spp.	15.6	20	25	10	0
Pestalotia sp.	9.4	20	12.5	0	0
Phaeoacremonium chlamydosporum	56.3	40	62.5	60	100
Phaeoacremonium sp.	12.5	10	12.5	20	0
Phomopsis sp.	28.1	0	50	30	66.7
Sphaeropsis sp.	28.1	20	37.5	10	66.7
Others	21.9	20	37.5	20	0
Undetermined	56.3	40	75	60	33.3

[a] Percentages were calculated on 31 total vines (TOT), 10 asymptomatic (ASYM), 8 showing symptoms of esca (ESCA), 10 aspecific symptoms (ASPE) and 3 apoplexy (APO).

occurred only in spongy decay tissue (B). A *Sphaeropsis* sp. was isolated both from the sectorial necrosis (A1) and from the underbark blackening (A4), while *Fusarium* spp. were found only in the latter area.

Fomitiporia sp. was present on vines with foliar symptoms of esca and on one apoplectic vine (Table 2), while *E. lata* and *P. chlamydosporum* were distributed at various level in all plants with (and without) foliar symptoms. Of these fungi, only *Fomitiporia* sp. was not found on vines without foliar symptoms and with aspecific symptoms.

Discussion

No substantial differences in types of wood deteriorations and in fungal occurence were noticed between foliar-asymptomatic plants and plants with aspecific symptoms.

Spongy decay, always with *Fomitiporia* sp., was found in most of the vines showing typical esca symptoms. *Fomitiporia* sp. seems to be able to produce spongy decay only on already necrotic tissues, but these necroses could be due to many factors, and could ever be non-parasitic. Necrotizing agents such as *P. chlamydosporum* and *E. lata* can certainly contribute to the development of spongy decay, but they are not indispensable. The occurence of spongy decay in plants with esca symptoms, and its absence from foliar-asymptomatic plants or plants with aspecific foliar symptoms, suggests that this type of deterioration is in some way related to the foliar symptoms. In that case it could be due to toxic metabolites produced by *Fomitiporia* sp., since the extent of spongy decay was always too restricted to interfere significantly with sap transport (Graniti *et al*., 1999). However, in view of the lack of spongy decay in two of the plants examined, other hypotheses must also be considered.

E. lata and *P. chlamydosporum* (previously named *Cephalosporium* sp. or *P. parasitica*) were isolated rather frequently from vines with esca symptoms and both are considered to be involved in the aetiology of the disease (Chiarappa, 1959; Larignon and Dubos, 1987; Minervini and Bisiach, 1988; Serra, 1995; Mugnai *et al*., 1996a). Both were present however in only one of the two vines that showed foliar symptoms of esca without spongy decay; in the second vine, the only fungus known to cause wood disease was *Sphaeropsis* sp.

E. lata was isolated only from necrosis originating in cane stumps, which suggests that the fungus is always present in the vineyard and can easily penetrate through such cuts. Its presence does not seem to be associated with any kind of foliar symptom. From the sectorial necrosis of the one vine with few stunted shoots on the dry cordon (symptoms in close association with eutypiose) sterile mycelium and a *Sphaeropsis* sp. were isolated.

P. chlamydosporum was found mainly in the rootstocks of plants both with and without esca foliar symptoms. We frequently isolated this fungus from blackish streaks (often concentrated around the pith forming a necrotic area) in apparently healthy rooted grafts but not from the plant material used for those grafts (unpublished data). This fungus probably penetrates through cuts or roots during the preparation and storage of rooted grafts. In a recent study, the fungus was isolated from canes in a vineyard with a high rate of esca, and spore trapping suggested an aerial mode of dissemination (Larignon *et al*., 2000). *P. chlamydosporum* was also isolated, though to a lesser extent, from the necrosis originating in the cane stumps and from the blackish streaks.

P. chlamydosporum and other related species have been associated with the decline of young grapevines in different countries (Mugnai *et al*., 1999), and pathogenicity tests on seedlings and rooted cuttings have satisfied Koch's postulates (Gubler *et al*., 1998). Nevertheless, *Phaeoacremonium* species can also behave as endophytes without damage to their host (Graniti *et al*., 1999). They are probably opportunistic fungi taking advantage of stressful conditions. In the present study, it appeared that *P. chlamydosporum* did not interfere with vine growth. However, the blackish halo around the pith may be the origin of the central necrosis often found in old vines. Necrosis probably extend in time from the rootstock to the rest of the plant whose vitality is waning because of its exploitation and normal ageing.

The apoplectic vines showed deterioration of the roots together with blackening of the rootstocks - largely colonized by *Sphaeropsis* sp. and *Fusarium* sp. - and emergence of new roots in the upper node. The slight presence of spongy decay, associated with a *Fomitiporia* sp., in the cordon of one of these plants was probably not responsible for its sudden wilting.

In conclusion, it is difficult to relate the foliar symptoms to the occurrence of particular pathogens. Certainly, the number of vines examined in the present study was too small to draw conclusions, most of all considering the wide variety of case histories and the extreme variability in symptom expression typical of esca. Further studies should be done for a better understanding of the fungi causing foliar symptoms and their role in causing esca; however, only the fulfilment of Koch's third postulate can definitively dispel all doubts still existing on esca aetiology.

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