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Wood discoloration and decay in grapevines with esca proper and their relationship with foliar symptoms

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Summary. From two Italian vineyards affected with esca proper, vineyard A (Borghi, FC, Emilia-Romagna) and vineyard B (Controguerra, TE, Abruzzo), located in different wine-growing areas and with different cultivars, 165 vines were collected that had shown the leaf symptoms of esca proper in the years before. Each vine was inspected and the cubic volume of the main symptoms in the trunk was measured: discoloration, including the various forms of dark necrosis, and decay. In each vine of both vineyards the extent of wood deterioration was compared with the severity of the leaf symptoms recorded in the years before the vines were cut down. The existence of a correlation between discoloration and decay in the wood colonisation process was explored. No correlation was found between the severity of the wood deterioration and the severity of the leaf symptoms. The typical leaf symptoms were found also on vines that had only wood discoloration without any decay. There was no correlation between wood discoloration and decay in vineyard A in which the vine wood was more degraded, with a greater incidence of decay. In vineyard B, however, in which the incidence of decay was less, a correlation between decay and discoloration was potentially found.

Key words: vinewood deterioration extent, *Phaeomoniella chlamydospora, Phaeoacremonium aleophilum, Fomitiporia mediterranea, Botryosphaeria obtusa*.

Introduction

The most common form of esca in the vineyard is that which is today termed esca proper, in which vines become affected internally with different types of wood deterioration, namely discoloration and decay, caused by different pathogens. Esca proper is today considered the combined result of two distinct diseases, a tracheomycosis caused by *Phaeomoniella chlamydospora* with possibly some species of *Phaeoacremonium* as well, and a white rot caused by *Fomitiporia mediterranea* (Surico *et al.,* 2006). Recently it has been established that *F. mediterranea* mainly invades the vine wood (Chiarappa, 1997; Graniti *et al.,* 2001), while other studies have ascertained that the main three esca fungi (*P. chlamydospora, Phaeoacremonium aleophilum* and *F. mediterranea*) can act independently in time and space, but that to achieve infective success they can also act in a certain order, or in combination (Sparapano *et al.,* 2000a; 2000b).

Esca proper is particularly harmful because it is very widespread, because of the direct loss in production it causes, and because measures to control or limit the disease are lacking (Di Marco *et al.,* 2000).

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The numerous studies on esca proper that have been carried out recently have shed much light on the complex aetiology of the disease (Mugnai *et al.,* 1996b; Surico *et al*., 2001) but many questions still remain unanswered. These include the actual process of wood deterioration, the antagonism and synergy of the fungi involved, the correlation between wood and leaf symptoms, and other internal and external factors that affect symptom expression.

In this work, the wood deterioration of vines affected with esca proper from two vineyards was identified and measured in order to look for a correlation between the types of wood deterioration and the leaf symptoms found. A preliminary attempt was also made to understand the process of colonisation of the vine wood, exploring the interrelation between the different types of wood deterioration while the wood was being invaded. Moreover the micro-organisms causing these types of wood deterioration have been isolated on nutrient media to corroborate the earlier findings reported in the literature.

Materials and methods

Location and plant material

The study was carried out in two vineyards located in different parts of Italy, vineyard A, at Borghi, province of Forlì-Cesena, Emilia Romagna, and vineyard B, at Controguerra, province of Teramo, Abruzzo. Vineyard A, aged 36 years, was established with cv. Sangiovese trained to the spur-pruned cordon system, and comprised 1526 vines, and vineyard B, aged 32 years, was established with the cv. Trebbiano d'Abruzzo trained to the tendone system, and contained 1460 vines.

Leaf symptoms

In 1994 esca proper incidence was 69.4% in vineyard A (cumulated incidence) and 11.4% in vineyard B (annual incidence). In both vineyards leaf symptoms were determined, calculating their severity as a percentage of the total area of the crown. Leaf symptom severity was determined in 1991–1994 in vineyard A, and in 1994 in vineyard B, on vines that were then cut down in 1995 to determine the extent of the wood deterioration of these vines.

Wood deterioration

To determine the extent of wood deterioration in affected vines, 111 vines with leaf symptoms from vineyard A and 54 vines from vineyard B were collected. The vines had an average trunk diameter of 10 cm, which corresponded to a trunk cross-section area of 78.5 cm^2. The trunks were cut into 10-cm-thick disks from the intersection of the branches to the intersection of the roots. The branches were examined separately. On each wood disk the percent area of each disk (upper and lower surface) affected with wood deterioration was calculated visually using a scale with 5% increments from 5 to 100%. The percent affected area of the two sides per disk was averaged and multiplied by the thickness of the disk (10 cm) so as to give the volume (cm^3) of each type of deterioration in that particular disk. The sum of the volumes of such deterioration in all the disks of each vine yielded the total volume of deterioration in that vine. The volume taken up by the various types of deterioration was divided into two classes depending on the fungal species associated with them (see Fungal isolation, next section): discoloration, which included pink discoloration, black streaking and darkening, central and sectorial dark necrosis, pith darkening, and decay. Of the two main types of deterioration, discoloration and decay, discoloration was divided into: small volume discoloration (SVD) if the volume was smaller than 5000 cm^3 large volume discoloration (LVD) if it was greater than 5000 cm^3; decay was divided into small volume rot (SVR) if the rot volume was smaller than 2000 cm^3, and large volume rot (LVR) if it was greater. On the basis of this division the vines were thus classed into 6 categories: SVD, LVD, SVD+SVR, LVD+SVR SVD+LVR, LVD+LVR.

Statistical analysis

Each vine was analysed statistically to detect a correlation between the volume occupied by discoloration or decay and the severity of the foliar symptoms in the years before the vine was cut down, and also to look for a correlation between the volume of discoloration and the volume of decay, using Pearson's correlation procedure at α=0.05, with SAS version 8.1 (SAS Institute, Cary, NC, USA).

Isolations

The fungi associated with the wood deteriorations were isolated from 30 vines in each vineyard following the method of Mugnai *et al.* (1996b). In each vine the most extensive wood deterioration areas were identified (decay, light sectorial discoloration, brown central discoloration, brownish pith) and from each of these types of deteriorated wood 30 fragments were collected (15 from the centre of the deterioration and 15 from the margin). With smaller-size deteriorations, black streaks and pink discolorations, 15 fragments were excised for each type of deterioration. A total of 2790 wood fragments were collected from vineyard A and 2655 from vineyard B.

The fungi isolated were identified on the basis of their morphological characteristics in culture.

The isolations of each fungus from each type of wood deterioration are expressed as the frequency, which was the number of wood fragments from each wood symptom colonised by each fungus, as a percentage of the total number of wood fragments analysed.

Results

Leaf symptoms

Neither wood discoloration volume nor decay volume was correlated with the severity of the leaf symptoms in the year or years before the vines were cut down in either vineyard (Table 1). In both vineyards a sizable proportion of vines cut down for examination in 1995 (which had all shown leaf symptoms in at least one of the years before) exhibited only discoloration without decay. The percentage of such symptomatic vines with discoloration but no decay was 46.2% in vineyard B (Fig. 2) and 7.2% in vineyard A.

Wood deterioration

The volumes for discoloration and decay (Fig. 1) differed between the vineyards. They were higher in vineyard A, where 50.4% of vines were in class LVD+LVR, and 33.3% in LVD+SVR, as compared with vineyard B, in which 5.5% of vines were in class LVD+LVR, and 7.4% of vines in LVD+SVR. In vineyard B 44.4% of vines were in class SVD and 37% in SVD+SVR (these classes, comprising less severely affected vines, were rare in vineyard A) Some vines only had discoloration (see the section Leaf symptoms, above). Vines in class SVD+LVR were scarce in both vineyards. Examination of the branches of all vines in both vineyards only detected black streaks..

Statistical analysis (Table 1) detected a tendential direct correlation (R=0.635) between the discoloration volumes and the rot volumes of vineyard B, but in vineyard A no such correlation was detected (R=0.238).

Isolations

The particular fungal species associated with the different types of wood deterioration were those already reported in previous studies (Mugnai *et al.,*

Table 1. Correlation between discoloration volume and decay volume and foliar symptoms severity in esca affected vines of Borghi (A) and Controguerra (B) vineyards.

Correlation	R[a]	
	Borghi	Controguerra
Discoloration volume - decay volume	0.238	0.635
Discoloration volume - foliar symptoms severity 1991	-0.052	-
Discoloration volume - foliar symptoms severity 1992	-0.187	-
Discoloration volume - foliar symptoms severity 1993	-0.001	-
Discoloration volume - foliar symptoms severity 1994	-0.050	0.245
Decay volume - foliar symptoms severity 1991	0.274	-
Decay volume - foliar symptoms severity 1992	0.382	-
Decay volume - foliar symptoms severity 1993	0.252	-
Decay volume - foliar symptoms severity 1994	0.190	0.167

[a] Statistical correlations were calculated by Pearson's correlation analysis (α=0.05); R = Pearson's correlation coefficient.

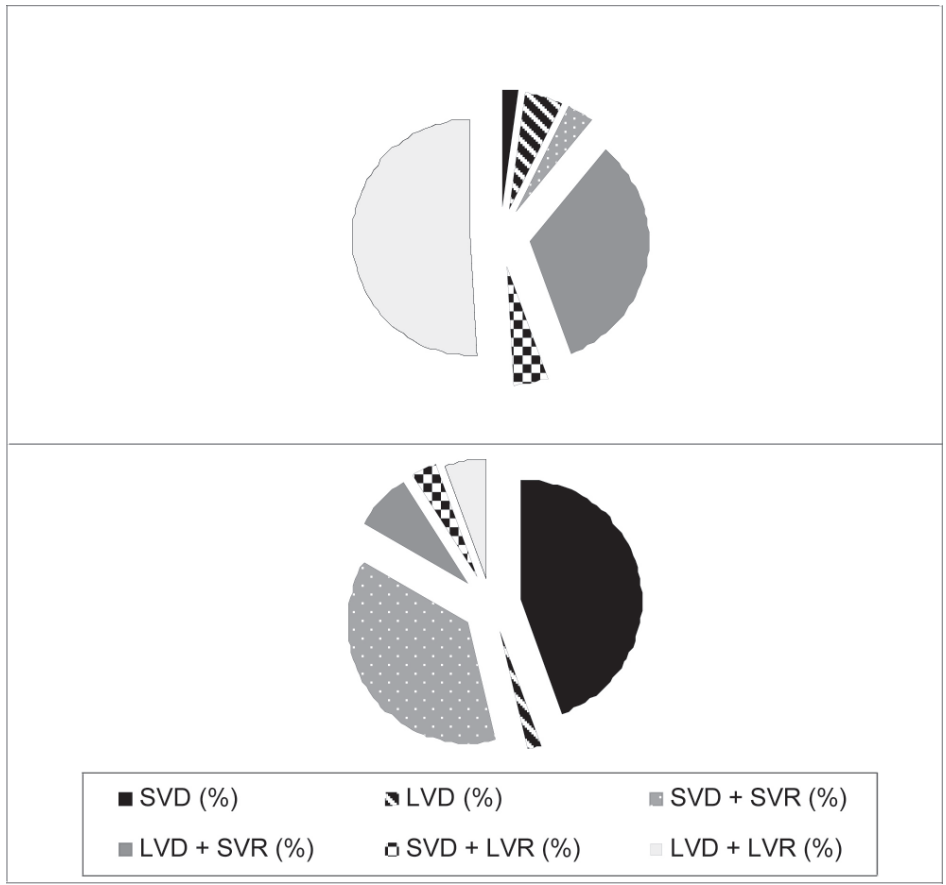

Fig. 1. Classification of esca affected vines of vineyard A (Borghi, top) and vineyard B (Controguerra, bottom) for type and extension of wood deterioration: Small Volume Discoloration, Large Volume Discoloration, Small Volume Discoloration+Small Volume Rot (Decay), Large Volume Discoloration+Small Volume Rot (Decay), Small Volume Discoloration+Large Volume Rot (Decay), Large Volume Discoloration+Large Volume Rot (Decay).

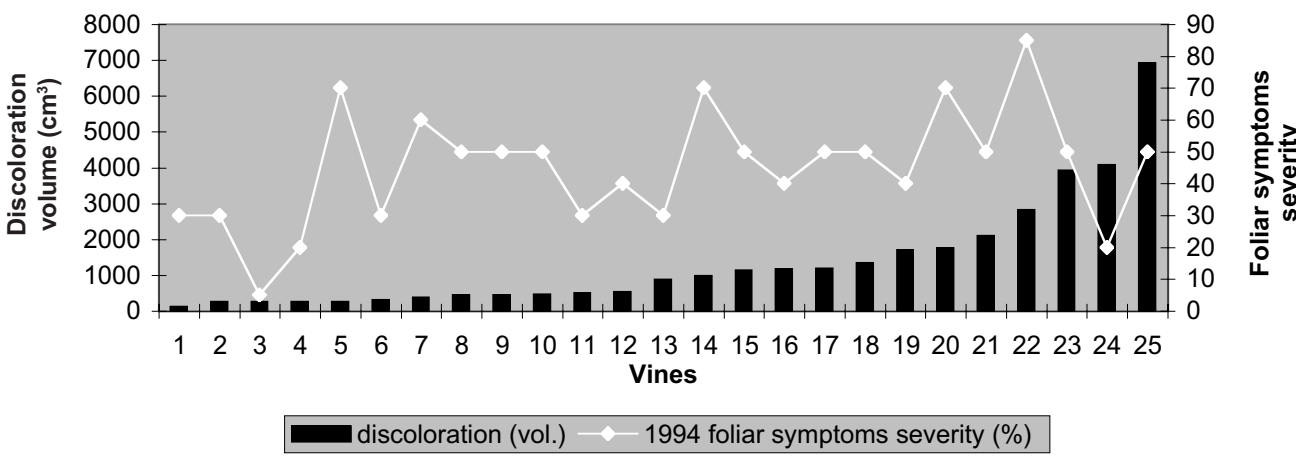

Fig. 2. Foliar symptoms severity in the year before cutting the vines (1994) and extent of wood discoloration in the trunk of vines showing no decay (Vineyard B).

1996b; Larignon and Dubos, 1997). The most common fungi and their isolation frequency were, for wood decay: *F. mediterranea,* 67.1% (vineyard A), 36.8% (vineyard B); and for discoloration of various kinds: *P. aleophilum* in light discoloured sectorial wood and brownish pith, 42.1 and 19.5% respectively in vineyard A, and 15.6 and 20.0% in vineyard B; *P. chlamydospora* in brown-discoloured central wood, in the black streaks and in the pink discoloured wood, 43.0, 37.8 and 35.5% respectively in vineyard A, and 53.3, 43.3 and 20.0% in vineyard B.

Botryosphaeria obtusa was commonly isolated with all types of wood deterioration in both vineyards, especially in the brown-discoloured pith (26.7%) and the brown-discoloured central wood (11.1%) of vineyard A, and in the brown-discoloured pith (19.6%) and decayed wood (11.8%) of vineyard B.

In total 1796 fragments out of 2655 from the wood samples of vineyard B were infected, and 2022 fragments from 2790 samples of vineyard A. There were however more fungal colonies than infected fragments because individual fragments were often colonised by more than one colony: consequently 1949 colonies were isolated from the 1796 infected fragments of vineyard B and 2197 colonies from the 2022 infected fragments of vineyard A.

Discussion

The lack of a correlation between the severity of the leaf symptoms and the severity of either type of wood deterioration, found in the present study, confirms previous findings on the erratic nature of leaf symptom manifestation. Many researchers have reported that vines affected with esca exhibit leaf symptoms of greatly varying intensity from one growing season to another, although the inner wood tissues were all the time affected with discoloration and decay (Mugnai *et al.,* 1996a; Surico *et al.,* 2000a; Calzarano *et al.,* 2001; Marchi *et al.,* 2006). One finding that deserves note constitutes the first experimental evidence of a hypothesis on esca advanced before, namely that vines even when they have only discoloration, and sometimes only slight discoloration, and no decay at all, can yet exhibit leaf symptoms of varying and indeed great severity, although the inner wood tissue of those vines is but little affected. This finding thus demonstrates that wood decay is not a necessary concomitant of the chlorotic and necrotic leaf symptoms typical of esca.

A comparison of the volumes in the vinewood occupied by discoloration and decay in both vineyards revealed that wood decay was much more extensive in vineyard A than in vineyard B. The lower levels of wood colonisation in vineyard B, even though the annual incidence of esca proper was more or less the same as in vineyard A, show that wood colonisation was not so far advanced in vineyard B as in vineyard A, where most of the vines had decayed wood. In vineyard B the correlation found between wood decay and discoloration suggests that the rot agent is favoured by necrosis and wood discoloration (this correlation disappears when the rot spreads to a large part of the discoloured wood, as in vineyard B). These findings thus confirm that decay is favoured when a number of different micro-organisms attack the wood in a certain succession (as suggested by Larignon and Dubos, 1987), although various researchers (Chiarappa, 1997; Sparapano *et al.,* 2000a) have left no doubt that such a concourse of other fungi is not necessary for *F. mediterranea* to colonise the vine wood. The onset of wood rot appears therefore as a natural part of wood deterioration, which may be favoured by the prior presence and colonisation of other fungi, including (as the isolation findings of the present two vineyards also make clear) those fungi causing tracheomycosis and wood discoloration.

Among the fungi isolated from deteriorated wood the role of *B. obtusa* is particularly uncertain because it was found at high levels in both discoloured and decayed wood in both vineyards. The occurrence of this fungus in esca-deteriorated vine wood typically associated with other microorganisms and its known ability to cause necrosis and decline in grapevine may well suggest that it has a role in wood deterioration (Cristinzio, 1978; Rovesti and Montermini, 1987; Di Marco and Draghetti, 1993a). Nevertheless the role of this fungus is at present still unclear and controversial (Larignon and Dubos, 2001; Larignon, 2005; Lecomte *et al.,* 2005a,b).

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