Phytopathol. Mediterr. (2000) 39, 206-210

Observations on the fungi associated with esca and on spatial distribution of esca-symptomatic plants in Apulian (Italy) vineyards

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Summary. The paper reports the results of observations on the fungi associated with deteriorated wood of esca affected vines and the spatial distribution of diseased plants in 21 vineyards located in Apulia (Southern Italy). Examination of over 43,000 plants revealed that the incidence of plants showing symptoms of esca was 12% (5-18%) in vineyards younger than 10 years and 22% (4-54%) in older ones. The most common deteriorations of the wood were a white rot of soft consistency and a dark brown discoloration of a hard consistency including longitudinal black streaks. Mycological analysis was carried out on 554 diseased vines from 5 vineyards. *Phellinus* sp. was frequently isolated from white rot, whereas *Phaeoacremonium* spp., *Botryosphaeria* spp. and *Eutypa lata* were more often isolated from dark brown tissues. Preliminary observations of maps of plants with symptoms of esca seem to indicate a tendency to aggregation, especially in young vineyards.

Key words: grapevine, *Phellinus, Phaeoacremonium, Botryosphaeria, Eutypa lata,* spatial distribution.

Introduction

Esca, considered for a long time a physiological decline of older grapevine plants, was recognised as a fungal disease only at the end of the last century (Ravaz, 1898). In the last ten years its severity has increased notably and the disease now occurs even in young vineyards.

Many aspects of esca have not yet been clarified, particularly which pathogens are really involved in its etiology. In the past, ligninolitic fungi, such as *Phellinus igniarius* (L.:Fr.) Quél. and *Stereum hirsutum* (Willd.) Pers., were believed to be the causal agents (Viala, 1926). Chiarappa (1959) first established a relationship between wood decay and black measles and suggested the involvement of a *Cephalosporium* sp. in the disease. More recently, an etiological plurality has been hypothesised. According to this view, the basidiomycetes would be opportunistic fungi exploiting the previous action of precursory microorganisms, such as *Phaeoacremonium* spp., *Eutypa lata* (Pers.:Fr.) Tul. & C. Tul. and *Botryosphaeria* spp. (Larignon and Dubos, 1987, 1997; Larignon, 1991; Mugnai *et al*., 1996a, 1999). However, Chiarappa (1997) demonstrated that *Phellinus igniarius* may act as a primary pathogen. All these findings induced Graniti *et al*. (1999) and Mugnai *et al*. (1999) to re-open the question on whether esca is a "disease complex" or a "complex of diseases".

This paper deals with the fungi associated with deteriorated wood and with the spatial distribu-

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tion of diseased vines in some vineyards in Apulia (Southern Italy).

Materials and methods

Twenty-one vineyards (4-50 years old) were selected as representative of different farming conditions. They included different combinations of cultivars (Italia, Panse precoce, Victoria, Pizzutella, Sangiovese, Malvasia nera, etc.) and rootstocks (140 Ru, 1103 P, 779 P, 157/11 C). In 1997 and 1998, vineyards were surveyed in summer (July to October) in order to record plants showing the typical symptoms of esca on the leaves and/or bunches, and to prepare detailed maps showing their location. Over 43,000 plants were examined in two years.

A sample of more than 100 plants showing the typical symptoms of esca was collected from each of five 15- to 32-year-old vineyards cv. Italia (table grape). The plants were sectioned lengthwise and the wood was examined for internal symptoms. The different types of wood deterioration were classified in accordance with Larignon (1991) and Mugnai *et al*. (1996b).

Small pieces of tissues were sampled from wood portions with prevalent internal symptoms for mycological analysis. Five tissue pieces per isolation were placed on malt-extract agar (MEA). Petri dishes were kept at 25±1°C in the dark, and the colonies developed within one week were singly transferred to fresh medium (MEA or potato-dextrose agar, PDA) for identification.

Results and discussion

The expression of symptoms in individual vineyards varied widely in the two years (Table 1). The average proportion of plants showing symptoms in at least one year was 12% (5-18%) in vineyards younger than 10 years and 22% (4-54%) in older ones.

Table 1. Percentages of plants showing symptoms of esca disease in the vineyards surveyed in 1997 and 1998.

Vineyard No.	Year of planting	No. of observed plants	Plants showing symptoms (%)	
			1997	1998
1	1948	2,304	54	16
2	1977	1,400	18	31
3	1966	1,599	9	28
4	1990	945	63	18
5	1979	1,482	8	19
6	1979	1,245	6	11
7	1982	4,752	4	ns[a]
8	1991	1,784	7	4
9	1991	1,218	5	2
10	1992	568	62	ns
11	1994	1,681	ns	8
12	1966	2,734	ns	27
13	1960	4,104	24	6
14	1973	3,267	24	23
15	1977	2,162	16	31
16	1977	2,310	15	ns
17	1977	2,688	4	ns
18	1978	1,884	19	44
19	1993	900	ns	23
20	1980	1,312	ns	9
21	1968	2,880	ns	18
Total		43,219	21	19

[a] ns = not surveyed.

Table 2. Distribution (% of plants) of different symptoms of esca disease in the vineyards surveyed.

Plant's age (years)	No. of vineyards	Symptoms			Apoplexy
		Only on leaves	Only on bunches	On both leaves and bunches	
≤ 10	6	58	32	9	1
> 10	15	82	2	9	7

The frequency with which different symptoms of esca disease were found was dependent on the age of the plants. Symptoms on bunches, not accompanied by symptoms on leaves, were particularly frequent in young vineyards, while apoplexy was more frequent in older vineyards (Table 2).

In a 18-year-old vineyard (cv. Italia grafted on 140 Ru), to be replanted, it was possible to check all the plants for internal symptoms. Seriously deteriorated wood was observed in 84% plants despite a low frequency (17%) of plants showing symptoms on the leaves or bunches. This result demonstrated the poor correlation between the severity of the disease in the wood and the expression of external symptoms on the plants in a given year.

Preliminary study of maps of plants with symptoms of esca seemed to indicate a tendency to aggregation, especially in young vineyards (Fig. 1). No clear conclusion could be drawn in most old vineyards because of the very high disease incidence, but a trend to aggregation was sometimes still obvious (Fig. 2). These results are in agreement with findings of Surico *et al.* (1999). In 1999,

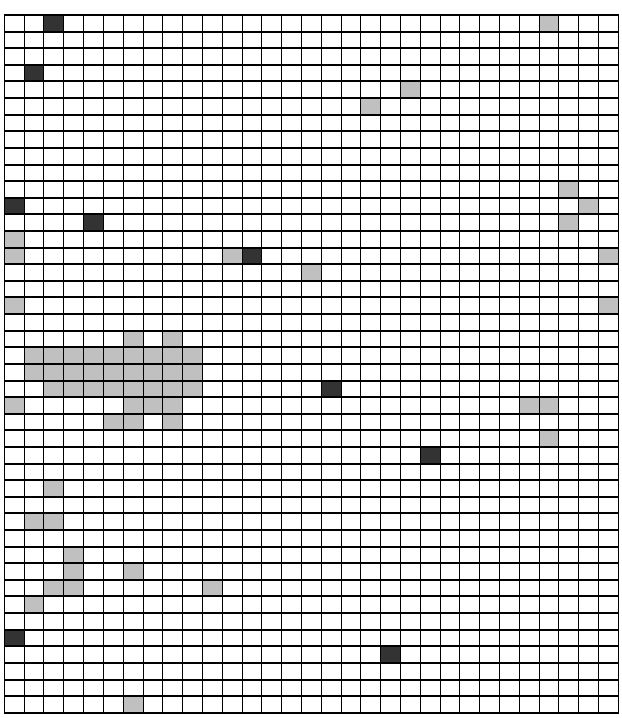

Fig. 1. Distribution of plants showing symptoms of esca (grey squares) and uprooted plants (black squares) in a 6-year-old vineyard cv. Italia grafted on 140 Ru (No. 9 in Table 1, surveyed in 1997).

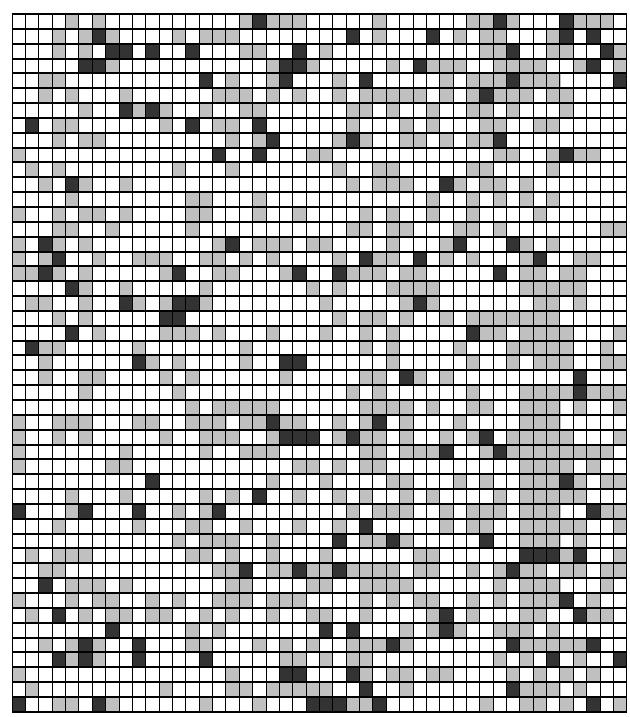

Fig. 2. Distribution of plants showing symptoms of esca (grey squares) and uprooted plants (black squares) in a 21-year-old vineyard cv. Italia grafted on 140 Ru. (No. 15 in Table 1, surveyed in 1998).

the survey was carried out for a third year. Cumulative data will be subjected to geostatistical analysis to obtain information on disease epidemiology.

In old vines, the most common alterations of wood were: white rot of soft consistency, often bordered by a black line (type 1) and a dark brown discoloration of a hard consistency including longitudinal black streaks (type 2), both arising from pruning wounds. In young vines, browning of tissues without softening and longitudinal black streaks were the prevalent symptoms and often arose from the grafting point. Occasionally, incipient white rot was observed in discoloured wood.

Phellinus (*Fomitipora*) sp. colonies were obtained at a very high frequency from spongy wood, less often from the surrounding black line and only occasionally from dark brown wood (Table 3). Additional fungi were isolated mostly from the dark lines and dark brown wood. Briefly, *Phaeoacremonium* spp., especially *P. chlamydosporum*, were detected only occasionally in spongy wood, but much more frequently in the surrounding black line and in dark brown wood. Different species of *Botryosphaeria*, mainly *Botryosphaeria obtusa* Schw., were isolated sporadically from spongy wood and more frequently from dark-brown tissue. Although the symptoms of Eutypa dieback were never observed in the vineyards surveyed, colonies of *E. lata* were obtained rarely from spongy wood but at a frequency as high as 25% from dark-brown wood (Table 3). Other fungi, mostly saprophytes, were observed in about 30% of isolations.

Results corroborate data previously reported (Larignon and Dubos, 1987, 1997; Bisiach *et al*., 1990; Mugnai *et al*., 1996a; Serra, 1999). In plants with the typical symptoms of esca, *Phellinus* sp. (*Fomitiporia*) is prevalent in the spongy wood, while *Phaeoacremonium* spp., especially *P. chlamydosporum*, *E. lata* and *B. obtusa* are prevalent in the black and dark brown wood.

Phaeoacremonium spp. are believed responsible for a specific syndrome on young vines indicated with different names (slow dieback, black goo, etc.) and it has also been suggested that they play a role in the early stage of esca (Graniti *et al*., 1999; Mugnai *et al*., 1999). The present study will be continued by focusing on the fungi associated with deteriorated wood in young vines, on their behaviour under nursery conditions, and on the possibility of their spreading with propagation materials. It aims at improving knowledge about the role of different microorganisms at an early stage of the disease and on suitable crop protection approaches.

Table 3. Frequency (%) of fungi isolated from deteriorated wood of 554 plants cv. Italia showing external symptoms of esca sampled in five different vineyards.

Alteration [a]	Isolation from	*Phellinus* sp.	*Phaeoacremonium* spp.	*Eutypa lata*	*Botryosphaeria* spp.	Others
Type 1	Spongy wood	89	10	5	6	22
	Black line	52	36	5	9	26
Type 2	Internal tissues	15	52	25	16	39
	Border tissues	4	55	12	15	24

[a] Type 1: spongy tissues delimited by a black line; type 2: dark brown tissues of a hard consistency and/or longitudinal black streaks.

Acknowledgements

Work supported by a grant from the Regione Puglia, the Italian Ministero delle Politiche agricole e forestali, and the European Union (Programma Operativo Plurifondo Regione Puglia, Misura 4.3.1) to the project "Alterazioni del legno della vite (escoriosi, mal dell'esca e imbrunimenti delle barbatelle)".

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