

## RESEARCH PAPERS

# Susceptibility to esca of various grapevine (*Vitis vinifera*) cultivars grafted on different rootstocks in a vineyard in the province of Siena (Italy)

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**Summary.** One vineyard at Castelnuovo Berardenga in the province of Siena (Italy) was surveyed from 1995 to 1999 to determine the incidence of esca. The vineyard had been established in 1982 using various cultivars (17) and rootstocks (6). In July and September of each year 19 columns, comprising 44% of the total number of vines, were inspected for esca, and symptom severity of diseased vines was recorded. At the end of the survey period, maps showing the cumulative incidence of esca were drawn and the incidence for each cultivar and for each cultivar/rootstock combination was calculated. Data expressed on a binomial scale were analysed with the  $\chi^2$  goodness of fit test. Esca incidence differed significantly among cultivars ( $P < 0.001$ ). The statistical analysis showed that the 17 vine cultivars fell into 4 groups with varying susceptibility to esca. The most susceptible cultivar was Semillon, the least susceptible Roussanne. A possible indirect role of the rootstock in determining cultivar susceptibility to esca also emerged from the surveys.

**Key words:** grapevine, esca, susceptibility, cultivar, rootstock.

## Introduction

Esca continues to be one of the most serious grapevine disorders of biotic origin in many parts of the world. Though it has been the subject of study for almost a century, many aspects of the disease are still unclear. Despite progress achieved in the last 15 years, the epidemiology and control of esca

are only some of the lines of research that are currently being pursued (Graniti *et al.*, 1999; Surico *et al.*, 2000; Di Marco *et al.*, 2000). Another question that merits further study is whether vine cultivars themselves differ in susceptibility to the causal agents of esca: the mitosporic fungi *Phaeo-monniella chlamydospora* and *Phaeoacremonium aleophilum* and the basidiomycete *Fomitiporia punctata* (Larignon e Dubos, 1997; Mugnai *et al.*, 1999). A better understanding of such varietal differences are expected to be of use when devising genetic improvement programmes for enhanced resistance to esca.

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At present grapevines in vineyards all over the world are, with rare exceptions, plants which through the practice of grafting combine the genetic heritage of different species. *Vitis berlandieri*, *V. riparia* and *V. rupestris* are the most widely-used species of American origin that have been crossed to produce the rootstocks used in viticulture today (Bavaresco, 1998).

It has long been known that some species of *Vitis* contain characters conferring resistance to numerous disorders of biotic origin. According to Marsais (1923) grapevines grafted on *V. rupestris*, richer in tannins than *V. riparia*, are more frequently affected by esca. Langcake (1981) reported that the stress metabolites resveratrol,  $\epsilon$ -viniferin,  $\alpha$ -viniferin and pterostilbene were important components endowing *V. riparia* and *V. vinifera* with resistance, natural or induced, to *Plasmopara viticola* (downy mildew) and *Botrytis cinerea* (grey mould). In studies on scions of Pinot bianco grafted on 1103P, SO 4 and Kober 5BB on highly calcareous soils, Bavaresco and Zamboni (1990) found that resveratrol levels in the leaves were lowest on 1103P rootstock, higher on SO 4 and highest on Kober 5BB. The availability of nutrient macroelements such as potassium and nitrogen, of which at least the latter is also determined by the rootstock used (Fregoni, 1985), seems to influence the levels of some of the stilbenes in the leaves (Bavaresco, 1993).

Studies of the differences in susceptibility between varieties of *V. vinifera* subsp. *sativa* are hampered by a very peculiar characteristic of esca, the discontinuity in symptom expression in diseased plants from year to year. A vine plant that begins to show clear signs of esca in a given year may thereafter not display any symptoms at all for one or more years, during which time it will seem perfectly healthy (Surico *et al.*, 2000). The reasons for this discontinuity are still unclear but both this and more generally the type of external symptoms produced may also be connected to the variety grown, and to the interactions between the cultivars and particular characteristics of the vineyard. Since some factors external to the plant-pathogen complex (e.g. rainfall and temperature) vary from year to year, it seems likely that any cultivar-linked differences in susceptibility would only be detected if data were cumulated over a number of years. At

present it is thought that 3 to 5 years of regular inspections are needed to form a reliable picture of the health of all the plants in a vineyard (Surico *et al.*, 2000). In this work the incidence and symptom severity of esca were analysed over a period of five years in one Tuscan vineyard to assess the susceptibility of different vine varieties to the disease, and the role of the rootstock in causing that susceptibility.

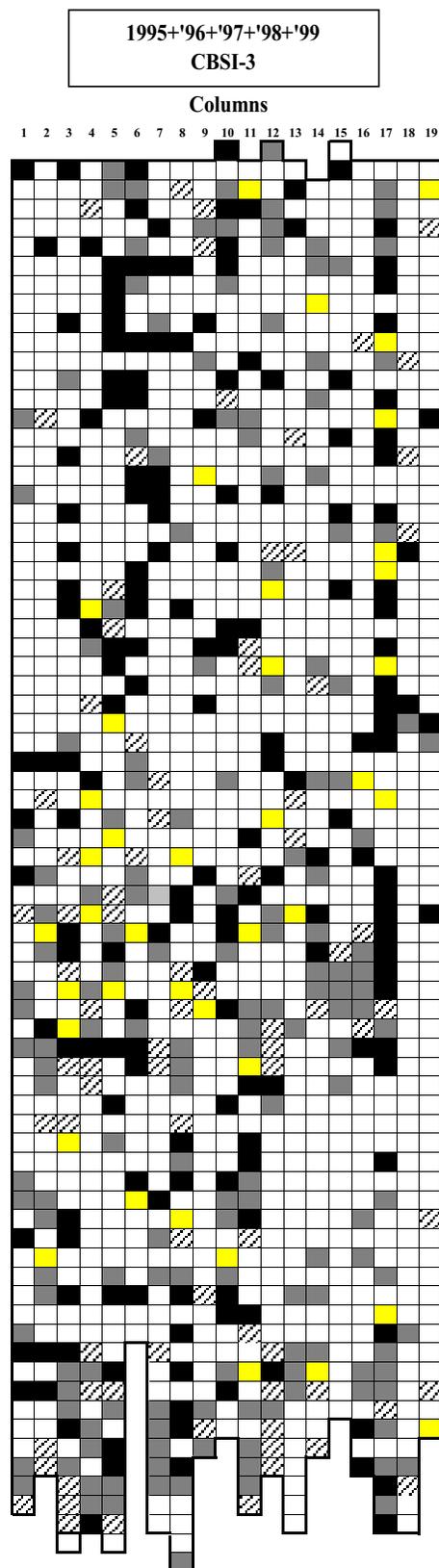
## Materials and methods

### Field surveys

Field surveys were carried out yearly from 1995 to 1999 in one experimental vineyard at Castelnuovo Berardenga (Azienda agricola San Felice S.p.A.), in the Province of Siena, Tuscany, Italy. The vineyard was established in 1982-1983 to determine the field performance of two vine cultivars, Sangiovese and Trebbiano, on various rootstocks, as well as to compare the adaptability to the Tuscan environment of 17 vine varieties grafted on 140RU rootstock. The vineyard area is approximately 1.7 ha and is bordered on the north, south and east by other vineyards, and on the west by an olive grove. The soil derives from calcareous marl from the Cretaceous, is rich in gravel, fairly deep, rich in calcium (27.8%) and rather poor in phosphorus, potassium, iron, magnesium and boron. The average slope of the experimental plots was 25%. The vineyard comprised 45 columns, of which 19 were inspected every year, and stretched from east to west following the slope. Each column began at the upper part of the slope with cv. Sangiovese on a range of rootstocks (average 28 plants per column), followed by cv. Trebbiano also on a range of rootstocks (average 28 plants per column), and ended with one of the 17 white-berried extraregional cultivars (two cultivars on two columns each) grafted on 140RU rootstock (average 70 plants per column). All the vines were outplanted with 2.8x1.4 m spacing, raised on free-standing trellises and pruned by simple Guyot with an average load of 11 buds per vine. The vineyard is located in an area characterised by hot and dry summers, with rainfall concentrated in the autumn-winter and spring periods.

To simplify, the highest part of the vineyard, planted with cv. Sangiovese, will be indicated as vineyard CBSI-1 (Fig. 1); that in the middle, planted with cv. Trebbiano as CBSI-2 (Fig. 1); and the





tomatic in any of the test-years were marked as healthy. In the course of these yearly inspections plants were sometimes found that did not revegetate after winter even though they had not shown any esca symptoms in the preceding growing season or seasons. Mostly there was no relation between these plants and disorders of a biotic origin other than esca, such as infection by *Armillaria mellea* or *Verticillium dahliae*, but the inner wood tissue of these plants did exhibit the typical alterations caused by esca agents, and for that reason they were marked as esca-diseased on the maps. All plants that were missing before July 1995 are also marked (Fig. 1 and 2).

### Statistical analysis

For each cultivar/rootstock combination and for each cultivar the cumulative esca incidence was calculated (Tables 2 and 3). Since the incidence data were dichotomous (plants either healthy or diseased), significant differences between cultivars or between cultivar/rootstock combinations were assessed with the  $\chi^2$  test. The data from CBSI-3, on the other hand, were processed with a cluster analysis according to Ward (Hau and Kranz, 1990; Fowler and Cohen, 1993) to reveal more clearly the differences and similarities between cultivars and groups of cultivars in their susceptibility to esca. This method has the advantage of minimising within-cluster dispersion. It was carried out with the computer programme Statistica from Stat Soft Inc. (1997). Generally the cluster procedure first measures the distance between individual cases, i.e. individual cultivars, represented on a Cartesian plane by the point of intersection of the X co-ordi-

Fig. 2. Vine cultivars planted in each column of vineyard CBSI-3 (one cv. per column):

1. Trebbiano toscano; 2. Malvasia lunga del Chianti; 3. Pinot bianco; 4. Pinot grigio; 5. Riesling italico; 6. Riesling renano; 7. Sauvignon; 8. Verdicchio; 9. Incrocio bianco fedit; 10. Incrocio 6.0.13; 11. Pinot bianco; 12. Malvasia istriana; 13. Chardonnay; 14. Vermentino bianco; 15. Canaiolo bianco; 16. Moscato bianco; 17. Semillon; 18. Roussanne; 19. Chardonnay. ■ = Plants exhibiting only chronic symptoms; ■ = plants with apoplexy; ▨ = plants that did not revegetate after winter and whose woody tissue presented signs of esca infection; □ = healthy plants; ■ = plants missing before 1995.

Table 1. Main agricultural characteristics of rootstocks used in the vineyards CBSI-1, CBSI-2 and CBSI-2 (Bavaresco, 1998; Fregoni, 1998)

Rootstock <sup>a</sup>	Geotropic angle <sup>b</sup>	Drought resistance	Moisture resistance	Resistance to active limestone (%)	Vigour
( <i>B.</i> × ( <i>Rup.</i> ))	40°–50°	-	-	-	-
( <i>Rip.</i> × <i>B.</i> )	60°–75°	-	-	-	-
420A ( <i>B.</i> × <i>Rip.</i> )	<i>a</i>	fair	poor	good (20)	low-moderate
K 5 BB ( <i>B.</i> × <i>Rip.</i> )	<i>a</i>	fair	fair	good (20)	high
SO 4 ( <i>B.</i> × <i>Rip.</i> )	<i>a</i>	poor	fair	fair (17)	moderate
1103 P ( <i>B.</i> × <i>Rup.</i> )	<i>b</i>	high	fair	good (20)	high
140 RU ( <i>B.</i> × <i>Rup.</i> )	<i>b</i>	high	poor	high (40)	very high

<sup>a</sup> *B.*, *Vitis Berlandieri*; *Rup.*, *Vitis rupestris*; *Rip.*, *Vitis riparia*.

<sup>b</sup> *a*, *b*, assumed to be of the same magnitude as the parental crossing.

-, unknown.

Table 2. Cumulative esca incidence in a vineyard planted with the cv. Sangiovese (CBSI-1) and Trebbiano (CBSI-2), on five different rootstocks and own-rooted. The incidence is the percentage of all plants that exhibited symptoms at least once in the five-year period 1995-1999.

Vineyard	Cultivar	Rootstock	No. standing plants in 1995	Cumulative incidence (%)	Average incidence (%)
CBSI-1	Sangiovese	140RU	80	16.2 <sup>a</sup>	15.3 <sup>c</sup>
		1103P	90	11.1	
		K5BB	87	24.1	
		SO 4	90	8.8	
		420A	90	17.7	
		own-rooted	75	13.3	
CBSI-2	Trebbiano	140RU	85	9.4 <sup>b</sup>	17.4
		1103P	90	24.4	
		K5BB	91	18.6	
		SO 4	91	8.7	
		420A	92	21.7	
		own-rooted	84	21.4	

<sup>a</sup> differences not significant between the 6 groups of Sangiovese ( $\chi^2 = 10.05$ ,  $P > 0.05$ ),

<sup>b</sup> differences significant between the 6 groups of Trebbiano ( $\chi^2 = 13.8$ ,  $P < 0.025$ ).

<sup>c</sup> differences in incidence between the two cultivars not significant ( $\chi^2 = 0.93$ ,  $P > 0.25$ ).

nate, representing the number of diseased plants and the Y co-ordinate, representing the number of asymptomatic plants for each cultivar at the end of the inspection period. It then joins these cultivars in first-order clusters, second-order clusters, third-order clusters, etc. on the basis of the selected similarity criterion (esca incidence). It ends when all cases have been placed in a cluster. The

procedure is therefore articulated in steps: initially the most similar cultivars (those with the most nearly identical disease incidences) are grouped together; subsequently ever less similar cultivars, i.e. those whose disease incidence is ever less similar. This means that when proceeding from the first-order clusters (Jiménez Diaz *et al.*, 1998) to the higher orders, the linkage distance (i.e. dissi-

Table 3. Cumulative esca incidence on 17 white-berried cultivars grafted on 140RU outplanted along 19 columns in vineyard CBSI-3. The incidence is the percentage of all plants that exhibited symptoms at least once in the five-year period 1995-1999.

Column	Cultivar	No. standing plants in 1995	Cumulative incidence (%)
1	Trebbiano	60	15.0 <sup>a</sup>
2	Malvasia b.l. Chianti	59	20.3
3	Pinot bianco	66	39.3
4	Pinot grigio	64	26.6
5	Riesling Italiceo	62	41.9
6	Riesling Renano	53	43.4
7	Sauvignon	62	24.2
8	Verdicchio	65	32.3
9	Incrocio bianco fedit 51	63	20.6
10	Incrocio 6.0.13	56	33.9
11	Pinot bianco	60	33.3
12	Malvasia Istriana	56	33.9
13	Chardonnay	66	12.1
14	Vermentino bianco	54	16.7
15	Canaiolo bianco	57	12.3
16	Moscato bianco	60	15.0
17	Semillon	58	63.8
18	Roussanne	69	8.7
19	Chardonnay	66	12.1

<sup>a</sup> The differences between the 17 white-berried cultivars are highly significant ( $\chi^2 = 113.8$ ,  $P < 0.001$ ).

milarity) between cases that become part of the same cluster increases at every step, until at the end all cultivars are englobed in a single most comprehensive cluster and the linkage distance is the greatest possible. A hierarchical tree plot was constructed with these data to show graphically the relations between the various cultivars.

## Results

At the end of the inspection period all cultivars and all cultivar/rootstock combinations were affected by esca though severity and incidence differed.

The data on esca severity were presented in a previous study (Surico *et al.*, 2000); those on disease incidence are shown in Tables 2 and 3.

### CBSI-1 and CBSI-2, cv. Sangiovese and Trebbiano

Though the samples were small, at least in the viticultural context in which the study was carried

out, differences in susceptibility were found. The cumulative incidence of esca at the end of the inspection period was 15.3% for Sangiovese (CBSI-1) and 17.4% for Trebbiano (CBSI-2) (Table 2). The overall frequency of healthy and diseased plants did not differ significantly between cultivars in the  $\chi^2$  test ( $P > 0.25$ ). There were however differences in susceptibility within both these cultivars when they were distinguished by their rootstocks. Cumulative incidences ranged from 8.8% for Sangiovese/SO 4 to 24.1% for Sangiovese/K5BB. For the cv. Trebbiano the lowest incidence was also on SO 4 rootstock, the highest was on 1103P. These differences within each cultivar due to rootstock were also tested with the  $\chi^2$  test, which revealed that incidence differences in the cv. Sangiovese were not related to the rootstock used ( $P > 0.05$ ), while by contrast incidence in cv. Trebbiano differed significantly by the rootstock ( $P < 0.025$ ). Trebbiano vines on 1103P and 420A and those that were own-rooted had disease incidences of 24.4, 21.7 and 21.4% respectively, more than double those of the same cv. on 140RU and SO 4 (9.4 and 8.7%).

### CBSI-3, white-berried cultivars

At the close of the inspection period there were strong and highly significant ( $P < 0.001$ ) differences in cumulative esca incidence between cultivars (Table 3). Cv. Semillon had the highest incidence (63.8%), cv. Roussanne the lowest (8.7%). Cumulative esca incidence in the other cultivars varied from 12.1% for cv. Chardonnay to 43.4% for Riesling renano. To bring out more clearly the differences and similarities between the various cultivars they were subjected to cluster analysis according to Ward method (Fig. 3, 4). Fig. 3 shows the cultivar clusters produced by the joining algorithm as a dendrogram, while Fig. 4 displays the within-cluster dispersion increments as increments of the linkage distance. In this graph the first fifteen steps of the joining algorithm are fairly small, going from 0 (joining cultivars with the same number of healthy and of diseased plants) to 15.8. With step No. 16, whereby cv. Semillon is joined to the cluster comprising cv. Pinot bianco (planted along two columns of the test plot), Riesling italiceo, Riesling renano, Verdicchio, Incrocio 6.0.13 and Malvasia istriana, the dissimilarities among the cultivars in the cluster become much greater, as shown by the increased linkage dis-

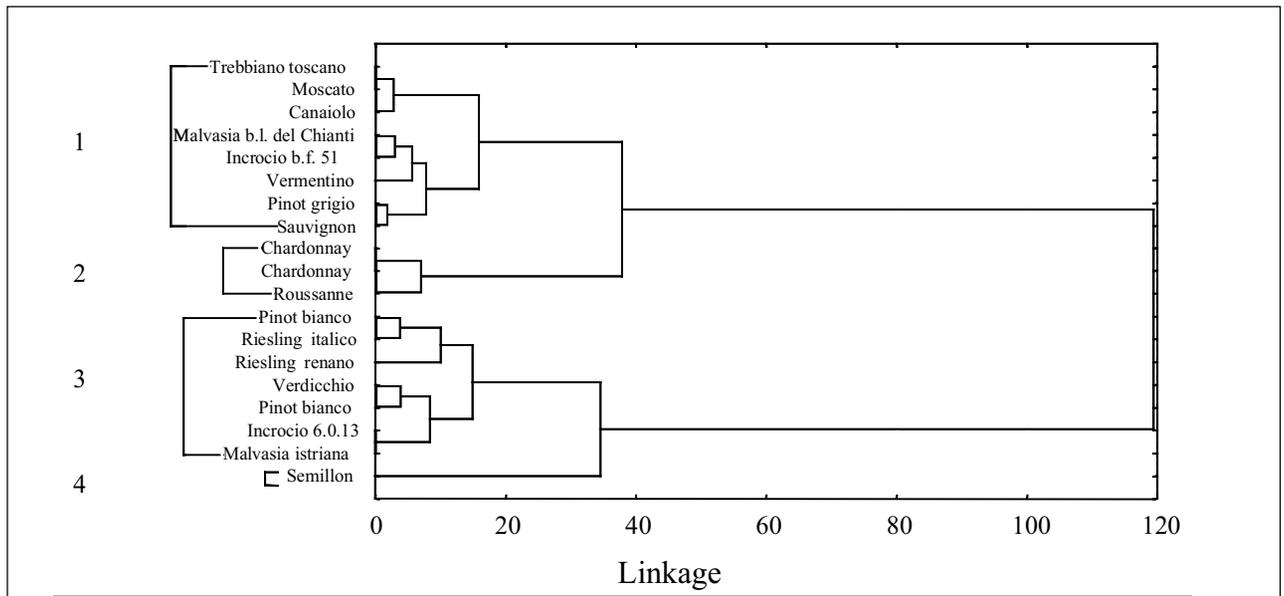


Fig. 3. Horizontal hierarchical tree plot of the 17 white-grape cultivars grown in vineyard CBSI-3. Amalgamation was determined on the number of healthy plants and esca-infected plants counted for each cultivar in 1999 at the end of the five-year inspection period. The cultivars Pinot bianco and Chardonnay were planted on two columns. The figure shows 4 clusters (identified as 1-4) which represent a possible grouping of the cultivars by their susceptibility to esca.

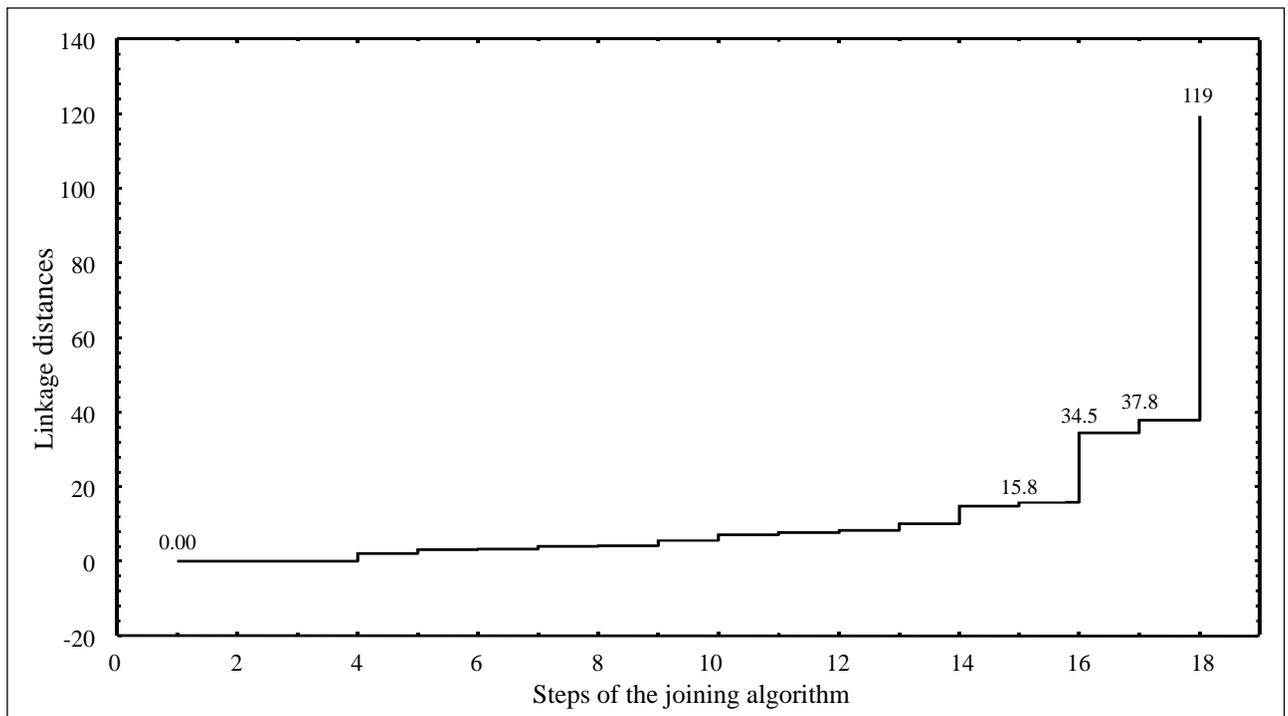


Fig. 4. Increase of the linkage distances (within-cluster dispersion) with the increase in the number of steps of the joining algorithm. Diagram expresses the same data as in Fig. 3.

Table 4. Number of vines inspected in vineyards CBSI-1, CBSI-2 and CBSI-3 in the period 1995-1999 that exhibited apoplexy or did not revegetate after winter dormancy although they had not exhibited any external esca symptoms in the preceding year or years.

Vineyard	Cultivar	Rootstock	No. standing vines in 1995	No. apoplectic strokes	No. plants dead over winter	Total dead plants
CBSI-1	Sangiovese	140RU	80	1	0	1
		1103P	90	1	0	1
		K5BB	87	2	1	3
		SO 4	90	1	1	2
		420A	90	3	0	3
		none	75	3	5	8
CBSI-2	Trebiano	140RU	85	1	1	2
		1103P	90	1	0	1
		K5BB	91	2	3	5
		SO 4	91	1	0	1
		420A	92	0	4	4
		None	84	2	5	7
CBSI-3	Trebiano	140RU	60	0	2	2
	Malvasia b.l. Chianti	140RU	59	3	4	7
	Pinot bianco	140RU	66	3	6	9
	Pinot grigio	140RU	64	4	7	11
	Riesling Italicco	140RU	62	3	6	9
	Riesling Renano	140RU	53	2	3	5
	Sauvignon	140RU	62	0	6	6
	Verdicchio	140RU	65	3	5	8
	Incrocio b. f. 51	140RU	63	2	5	7
	Incrocio 6.0.13	140RU	56	1	1	2
	Pinot bianco	140RU	60	4	6	10
	Malvasia Istriana	140RU	56	3	9	12
	Chardonnay	140RU	66	1	4	5
	Vermentino bianco	140RU	54	2	4	6
	Canaiolo bianco	140RU	57	0	1	1
	Moscato bianco	140RU	60	1	3	4
	Semillon	140RU	58	7	2	9
	Roussanne	140RU	69	0	4	4
Chardonnay	140RU	66	1	3	4	

tance, which goes up to 34.5. With step No. 17, incorporating cv. Chardonnay, likewise growing along two columns of the test plot, and cv. Roussanne into the cluster comprising cv. Trebbiano toscano, Moscato, Canaiolo, Malvasia toscana, Incrocio bianco fedit 51, Vermentino, Pinot grigio and Sauvignon, the linkage distance between cultivars is 37.8. With the last step, No. 18, the union of the two distinct clusters that were formed by steps No. 16 and 17 increased the linkage distance to 119.

## Discussion

In CBSI-1 and CBSI-2 the susceptibility of the vine plant to esca appeared to be related to the type of rootstock used for at least some cultivar/rootstock combinations. The combinations Sangiovese/SO 4 and Trebbiano/SO 4 had the lowest esca incidence. The SO 4 rootstock confers a middle to high vigour on the vine plant, low drought resistance and a fairly good resistance to waterlogging. If the rootstock affects the plant's resistance/sus-

ceptibility to esca, it must be assumed that in the test plots excess water occurred for the entire duration of the inspection period, so that plants with middling vigour and fair resistance to waterlogging expressed esca leaf symptoms less frequently. In this connection it has recently been suggested (Corti and Cuniglio, 1998) that esca incidence in some vineyards in Chianti (Tuscany, Italy) could vary with the depth of the *gley* horizons, which lead to waterlogging when they are near the surface. The conditions of stress thus created with prolonged asphyxia and phytotoxic  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  ions would make the vine more susceptible to the causal agents of esca. With other cultivar/rootstock combinations, however, the rootstock seems to be less important than other factors, internal or external to the plants. For example, cv. Trebbiano grafted on rootstock 1103P had a cumulative esca incidence of 24.4%, more than twice that of cv. Sangiovese on the same rootstock (11.1%). Moreover, the cumulative esca incidence, at least on cv. Trebbiano, varied strongly even among rootstocks that were culturally and above all ontogenetically similar (Table 1). The combinations Trebbiano/1103P and Trebbiano/140RU for example had cumulative disease incidences of 24.4 and 9.4% respectively. This clearly indicates different interactions between a cultivar and similar rootstocks. It is in any case difficult to assess the effect which a rootstock has on esca symptom development, whether for the chronic or acute form of the disease. In a previous study (Surico *et al.*, 2000) it was reported that cool rainy summers favoured chronic esca, and hot dry summers acute esca. If then drought with high temperatures favours apoplectic strokes, it is to be expected that vine plants grafted on rootstock that confer drought resistance (1103P and 140RU, but also free-standing vines) would exhibit a lower incidence of such strokes; whereas under the same weather conditions on SO 4, conferring only poor drought resistance, the incidence of apoplectic strokes would rise. During the five-year inspection period apoplexy was not very common, killing 11 vines (2.1%) in CBSI-1, 7 (1.3%) in CBSI-2 and 42 (3.9%) in CBSI-3. (Table 4). If these apoplectic vines are added to those vines that failed to revegetate in spring though without any esca symptoms the previous year, the number of plants dead from esca increases to 18 (3.5%), 20 (4.1%)

and 115 (9.9%) for CBSI-1, 2 and 3 respectively. A breakdown by rootstock of CBSI-1 and CBSI-2 vine mortality revealed that 39% of dead vines (whether from apoplexy or failure to revegetate in spring) were own-rooted (53% Sangiovese, 47% Trebbiano), 21% were grafted on K5BB, 18% on 420A, 8% on 140RU, 8% on SO 4 and 5% on 1103P. It is interesting to compare the percent mortality of own-rooted plants with that of plants grafted on 140RU and especially 1103P. If apoplexy and failure to revegetate in spring is attributed to a water transport deficit in a system that is deteriorated by esca, it is tempting to postulate that vines on rootstocks that do not confer drought resistance are more subject to apoplexy even under non-pathological conditions. Clearly this was not the case in the vines examined here. Vines grafted on SO 4, with low drought resistance, and those grafted on 1103P, with high drought resistance, had much the same rate of apoplexy, while that in own-rooted vines, also with high drought resistance, was much higher.

The behaviour of the 17 white-berried varieties grafted on 140RU and outplanted in CBSI-3 was fairly clear-cut. Cv. Semillon, which was the most frequently infected, with a cumulative esca incidence of 63.8%, grew on column 17 and was flanked on one side by cv. Moscato Bianco (column 16) and cv. Canaiolo (column 15), which had a cumulative incidence of 15% and 12.3% respectively, and on the other by cv. Roussanne on column 18 and cv. Chardonnay on column 19, with a cumulative incidence of 8.7% and 12.1% respectively. Since the total distance between these five columns was only about 11.2 m, it seems fair to assume that growing conditions were the same for all of them, and consequently that cv. Semillon was more susceptible to esca than the other four cultivars. This assumption is corroborated by the fact that those cv. grown on 2 columns, Pinot bianco on columns 3 and 11, and Chardonnay on columns 13 and 19, had incidences very similar between columns, and, in the case of Chardonnay, identical. The level of esca incidence therefore varied with the cultivar planted. Cluster analysis for its part detected 4 clusters containing the following cv.: 1. Chardonnay and Roussanne (average incidence 10.97%); 2. Trebbiano, Moscato, Canaiolo, Malvasia bianca lunga del Chianti, Incrocio bianco fedit 51 (Gar-

ganega x Malvasia bianca lunga del Chianti), Vermentino, Pinot grigio, and Sauvignon (average 18.84%); 3. Pinot bianco, Riesling renano, Riesling italo, Verdicchio, Incrocio 6.0.13 (Pinot bianco x Riesling renano) and Malvasia istriana (average 37.45%); and 4. the single cv. Semillon (incidence 63.8%).

Surico *et al.*, (2000) showed that esca incidence in a vineyard, measured by counting all the plants that showed esca symptoms in at least one season during a three-year observation period, may be influenced by a wide range of environmental factors, such as rainfall, air temperature, vineyard slope, soil type, sun exposure, etc. The data presented in the present study were not subject to such factors since they all came from what was effectively one vineyard, and were therefore an objective measure of the susceptibility/resistance to esca of individual vine cultivars or cultivar/rootstock combinations. Of course this does not mean that a given cultivar may not show a different resistance to esca when grown in a different environment. For example, cv. Trebbiano had a low incidence of esca infection in CBSI-2 and CBSI-3 but a high incidence in another environment (Vercesi, 1988). This suggests that the susceptibility of vine genotypes to esca depends on environmental and genotype factors, or on combinations of less susceptible genotypes. These more resistant genotypes would be the preferred starting point for breeding programmes against esca.

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