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Esca symptoms appearance in *Vitis vinifera* L.: influence of climate, pedo-climatic conditions and rootstock/cultivar combination

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Summary

This study investigated the appearance of esca symptoms in relation to environmental factors and the rootstock/cultivar combination in an experimental setting between 2004 and 2009. Among the common genotypes showing susceptibility to the esca disease, four cultivars were considered: 'Cabernet Sauvignon', 'Sangiovese', 'Trebbiano Toscano' and 'Chardonnay'. These cultivars were studied own-rooted and in combination with two rootstocks: Kober 5BB and 1103 Paulsen. The difference in susceptibility of cultivars to esca appeared negatively related to the graft. No clear relation was found between esca appearance and environmental factors. Moreover, an unexpected discordance between esca incidence percentage and mortality rate was observed.

K e y w o r d s : *Vitis vinifera* L., esca disease, environmental conditions, rootstock.

Introduction

Vitis vinifera L. is strongly affected by esca, a multiple fungal syndrome causing serious economic problems for grapevine cultivation. (Hofstetter *et al*. 2012). Esca is a grapevine trunk disease occurring in adult plants aged 10 years or more and can manifest itself in two ways: a slow evolving form that is recognizable by visible foliar symptoms (chronic esca) or an apoplectic form (acute esca) that kills the plants within a few days (Mugnai *et al*. 1999). A peculiar characteristic of esca is the discontinuous expression of chronic symptoms in diseased plants from year to year (Mugnai *et al.* 1996, Surico *et al.* 2000, Calzarano *et al.* 2001, Christen *et al.* 2007), generally followed by death from apoplexy. As a consequence of the discontinuous expression of symptoms it is not possible to know when a plant becomes infected. Despite progress in recent years on epidemiology (Surico *et al.* 2000, Surico *et al*. 2006, Van Niekerk *et al.* 2011) and control of esca (Di Marco *et al.* 2000, Di Marco *et al.* 2011), several aspects of the disease remain unclear.

Esca incidence may be influenced by several pedo-climatic factors, such as rainfall, air temperature, vineyard slope, soil type, and sun exposure. In particular it has been observed that cool rainy summers favour the development of chronic esca, while high temperatures associated with drought promote acute esca (Surico *et al.* 2000). Recently, Van Niekerk *et al.* 2011 showed that the incidence and symptom profiles of the pathogens varied greatly between different climatic areas. This indicates that rainfall and temperature influence not only the distribution of pathogens but also their symptomatology.

Esca incidence may be influenced by the different susceptibility of cultivars. Some studies classify 'Cabernet Sauvignon' as a susceptible cultivar because of the high percentage of symptomatic vines observed in different climatic conditions and generally in mature vineyards. In contrast, Chardonnay has been classified as less susceptible to the esca disease (Christen *et al.* 2007, Borgo *et al.* 2008, Andreini *et al.* 2009, Andreini *et al.* 2013). The different susceptibility to esca among cultivars also appears to be affected by the rootstock combination (Marchi 2001). Thus the evaluation of genotypic characteristics is a valuable tool for genetic improvement programmes aiming to enhance cultivar resistance to esca. Beginning thirty years ago Kober 5BB (*V. riparia* X *V. berlandieri*) was the most commonly used rootstock in Italy. In the last ten years the areas planted with Kober 5BB were reduced in favour of 1103 Paulsen (*V. rupestris* X *V. berlandieri*), a rootstock tolerant to drought conditions and poor soils. Currently the 1103 Paulsen occupies about 30 % of winegrowing areas in relation to changes in vineyard management (in particular an increase in dense systems with over 5,000 vines per ha) and to climatic changes, mainly higher frequency of drought seasons (Tosi 2011). Despite the key role of rootstocks in conferring agronomical traits to cultivars (*i.e*. vigor and tolerance to biotic or abiotic stress), studies on the relationship between rootstock and esca disease are still rare. Recently, Andreini *et al.* 2013 carried out the first study to evaluate the effect of rootstock on the budbreak of vines affected by esca. Results showed that vines grafted on Kober 5BB were characterized by a significant delay in bud break.

The present study aimed to investigate the different susceptibility to esca disease of several cultivars of grapevine in relation to climatic and environmental conditions and rootstock/cultivar combination.

Material and Methods

E x p e r i m e n t a l s i t e a n d p l a n t m a t e r i a l : This study was carried out in a grapevine (*Vitis vinifera* L.) experimental field of the Department of Agriculture, Food

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and Environment (University of Pisa) located in the Tuscan coastal area (altitude 6 m, 43°02' N, 10°36' E). Vines were grafted or left not grafted in 1980 and planted in 1981. Since 2004 research at this experimental field site has been focused on the prevention and cure of esca disease (MESVIT, ARSIA-Toscana-Italy). Cultivars 'Cabernet Sauvignon', 'Sangiovese', 'Trebbiano Toscano' and 'Chardonnay' were analysed between 2004 and 2009. Each cultivar was grafted on 1103 Paulsen (1103P), Kober 5BB (K5BB) rootstocks and own rooted (35 plants/rootstock/cultivar). Vines, trained to a high free cordon, were planted at 3.0 x 1.0 m distance in 9 longitudinal rows. Excluding the two most external rows, a randomized complete block with four replicates arranged transversally to the length of the field was used. The soil was uniform across the experimental plot (42 % sand, 37 % silt and 21 % clay, pH 8). In the vineyard, all cultural practices (pruning, irrigation, fertilization and plant protection) were undertaken on the same date and in the same way for all cultivars.

Observation on esca symptomatology: At the beginning of this study, the total number of vines was 264 out of the 420 vines initially planted in 1981. The observed reduction in number vines was due to several causes including esca. During the experimental period a variable number of vines (grafted and non grafted) were examined, from 264 to 196, and the rate of mortality for each cultivar was calculated.

Every year, during the growing season (June-September), the incidence of esca disease was recorded from fruit set: (Stage 71: young fruits begin to swell) to harvest time (Stage 89: berries ripe). The phenological stages were identified by BBCH scale (Lorenz *et al.* 1994).

Each vine was examined on the basis of external esca symptoms, distinguishing between 'symptomatic' and 'non-symptomatic' vines. Symptomatic vines included both chronic and acute esca symptoms. The symptoms of chronic esca on leaves appeared slowly and consisted of light green color or chlorotic and rounded or irregular spots between the veins or along the leaf margins which usually spread outward to the distal part of the shoot (Fig. 1a). The acute symptom (apoplexy) appears quickly, affecting the whole vine with a total wilt, and is typically followed by death of the vine (Fig. 1b). Non-symptomatic vines never showed symptoms during the experimental trial. At the end of annual inspections the following parameters were determined: i) **incidence**: percentage of symptomatic vines observed each year; ii) **cumulative incidence**: total percentage of incidences recorded during the previous years, according to Marchi *et al.* (2006).

Soil analysis: The soil analysis was carried out in 2007 by collecting samples at 30 cm depth next to control vines and vines affected by esca. Granulometric composition, pH, total carbonate, calculated as $CaCO_3$, and exchangeable K were determined according to the MAAF (1994). Available P was determined spectrophotometrically on soil extracts according to Olsen *et al.* (1954). Organic carbon was determined by dry combustion (induction furnace 900CS, ELTRA). Total N determination was made by the Kjeldahl procedure after acid digestion (Bremner and Mulvaney, 1982). Cation exchange capacity (CEC) was

Fig. 1: Symptoms of esca: **a**) 'chronic' (light green or chlorotic leaves, rounded or irregular spots between the veins or along the leaf margins); **b**) 'acute' (quick decline of whole plant starting with leaf roll).

determined according to Bascomb (1964). Enzyme activities were assayed on fresh sieved soil stored at 4 °C, within 1 week from sampling. The hydrolysis rate of fluorescein diacetate (FDA-H) was estimated as reported by Swisher and Carroll (1980). Dehydrogenase activity was determined by a colorimetric method (Casida *et al.* 1964)

Statistical analysis: Significant differences among cultivars and among cultivar/rootstock combinations were evaluated using the χ^2 test (Cochran 1952).

Results

Discontinuous expression of esca symptoms: Generally the symptoms appeared between June and July during fruit set (Stage 71 of BBCH) with a significant increase in July when the berries developed colour (Stage 81-83 of BBCH). However esca incidence can increase up to the end of August. During the six year period (2004-2009) the percentage of vines that showed

esca symptoms in alternating years was similar among cultivars. The percentages ranged between 25 and 30 % for 'Chardonnay' and 'Cabernet Sauvignon' and 40 % for 'Trebbiano Toscano' and 'Sangiovese' (Fig. 2). The cultivars differed in whether the vines showed esca symptoms for only one year or for consecutive years. 'Chardonnay' was characterised by over 60 % of vines showing symptoms for only one year, while vines of 'Cabernet Sauvignon' generally showed esca disease in consecutive years (about 50 %). Only 'Cabernet Sauvignon' vines showed esca symptoms for five and six consecutive years (25 %). 'Chardonnay' and 'Trebbiano Toscano' showed a low percentage (about 10 %) of symptomatic plants for 2-4 consecutive years.

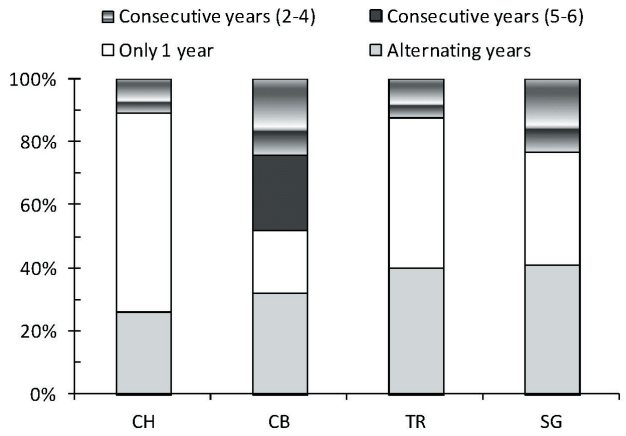

Fig. 2: Percentages of symptomatic vines determined in relation to the number of years during which esca symptoms appeared between 2004 and 2009. 'Chardonnay' (CH), 'Cabernet Sauvignon' (CB), 'Trebbiano Toscano' (TR) and 'Sangiovese' (SG).

Effect of environmental factors on esca incidence: From 2004 to 2009 the experimental site was characterised, on average, by annual precipitation of 880±187 mm, mainly concentrated during the autumn-winter season (data from ARSIA) and by hot summers with an average minimum and maximum temperature of 15.7 °C ± 0.6 and 28.3 °C ± 0.6, respectively. In particular, 2009 was characterised by a strong decrease in rainfall during the fruit growth period (June-August), when only 26 mm of precipitation was recorded (Tab. 1). At vineyard level the coefficient of determination (R^2) indicated that 64 % of variation in esca incidence was accounted for by variation in rainfall from June to August (during phenological stages 71-83). The maximum temperature during the period from June – August was also not correlated with esca appearance (R^2 = 4 %) (Tab. 1).

Results from the soil analysis showed that the physical characteristics, the availability of mineral elements (C, N, P, K) and the parameters related to soil microbial activity (FDA and TPF) were homogeneous across the vineyard and were ranged within an interval of values considered sufficient for normal development of the plants (Tab. 2). As a consequence a relationship between soil characteristics and esca fluctuation in the vineyard was not found.

Susceptibility of cultivars to esca: At the end of the 6-year period the examined cultivars showed significant differences in esca susceptibility estimated based on expression of symptoms (Fig. 3): high in 'Cabernet Sauvignon', moderate in 'Sangiovese' and 'Trebbiano Toscano', and low in 'Chardonnay'. Considering the highest and lowest susceptibility to esca, 'Cabernet Sauvignon' and 'Chardonnay' showed an average incidence of 45 % and 8 %, while an intermediate susceptibility was observed on 'Sangiovese' (24 %) and 'Trebbiano Toscano' (30 %). The cumulative incidence reached 70 % in 'Cabernet Sauvignon' and in 'Trebbiano Toscano', 58 % in 'Sangiovese' and 36 % in 'Chardonnay'.

By 2008 the mortality rate was below 10 %, but it reached 19.3 % during the last year of observation (Tab. 3). This increase in mortality in 2009 was due to a three to four fold increase in mortality of 'Chardonnay' and 'Sangiovese' vines compared to previous years.

Table 1

Total rainfall (mm) and average maximum temperature (°C ± s.e.) recorded from June to August. Percentage of symptomatic vines observed each year (incidence of esca) recorded at the end of growing season (September). Data collected during a five year period (2005-2009)

Year	June-August		September
	Total rainfall	T. max.	Incidence (%)
2005	87.2	29.0 ± 0.3	27
2006	56.9	28.6 ± 0.4	18
2007	72.4	27.4 ± 0.3	25
2008	79.4	28.1 ± 0.3	23
2009	26.2	28.7 ± 0.3	19

Rainfall *vs.* incidence: $R^2 = 0.64$; $y = 0.128x + 14.153$.
T. max. *vs.* incidence: $R^2 = 0.04$; $y = -1.2214x + 57.038$.

Table 2

Physical, chemical and microbiological characteristics of soil determined on samples collected at 30 cm depth across the vineyard at the base of control vines and vines clearly affected by esca symptoms

Soil Parameter		Control		Esca	
$g \cdot kg^{-1}$	Sand	387.1	± 28.7	375	± 53.5
	Silt	290.9	± 19.2	284	± 11.4
	Clay	322.0	± 24.4	341	± 46.6
	pH	8.0	± 0.14	8.0	± 0.16
%	Limestone	2.8	± 0.4	2.4	± 0.9
	C organic	1.4	± 0.6	1.5	± 0.6
‰	N total	1.7	± 0.5	1.7	± 0.4
	C/N	8.3	± 1.1	8.7	± 1.3
meq/100g	C.E.C	17.2	± 1.2	17.4	± 1.7
	K exch	304	± 188.0	312	± 207.0
$\mu g \cdot g^{-1}$	P assimil.	58	± 27.6	60	± 33.0
	FDA	197	± 93.3	184	± 82.9
	TPF	2.1	±1.2	2.0	± 1.5

Values are means ± standard error of 4 replicates.
Differences between "Esca" and "Control" values are not statistically significant.

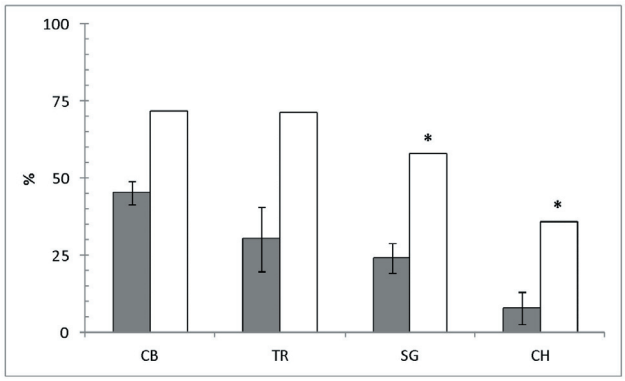

Fig. 3: Mean percentage of symptomatic vines (incidence) from 2004 to 2009 (■) recorded each year on 'Cabernet Sauvignon' (CB), 'Trebbiano Toscano' (TR), 'Sangiovese' (SG), 'Chardonnay' (CH). The percentages of cumulative incidence (□) observed in 2009 are reported. Asterisk denote statistically significant differences in cumulative incidence between the cultivars ($\chi^2 = 21.40$, by $P < 0.001$).

Table 3

Number of vines present at the start of trials (2004), and at the end of 2008 and 2009. Mortality rate (%) of Chardonnay (CH), 'Cabernet Sauvignon' (CB), 'Trebbiano Toscano' (TR), 'Sangiovese' (SG) was determined for two periods: 2004-2008 and 2008-2009

	Number of vines			Mortality rate (%)	
	2004	2008	2009	2004-08	2008-09
CH	90	81	57	10.0	29.6
CB	44	39	36	11.4	7.7
TR	45	42	37	6.7	11.9
SG	85	81	66	4.7	18.5
Total	264	243	196	8.0*	19.3*

* Mean of mortality rate of cultivars.

Effect of grafting process and rootstocks on esca incidence: For each cultivar the contribution of grafted and own rooted vines to symptomatic (Fig. 4a) and dead vines (Fig. 4b) was calculated. The own rooted vines allowed us to observe the genotypic characteristics of 'Chardonnay', 'Sangiovese', 'Cabernet Sauvignon' and 'Trebbiano Toscano'. A significant correlation between the percentage of symptomatic vines and the mortality rate was found in 'Chardonnay' and 'Sangiovese' (Fig. 4). 'Chardonnay' and 'Sangiovese' own rooted showed the lowest incidence of symptomatic vines (< 20 %) and also the lowest mortality rate (about 25 %). In contrast, 'Trebbiano Toscano' own rooted was characterised by both the highest esca symptoms appearance (44 %) and highest mortality (72 %). Interestingly, 'Cabernet Sauvignon' own rooted showed a different behaviour: a low incidence of esca appearance (25 %) corresponded to a high rate of vine mortality (66 %). Statistical analysis (χ^2 test) showed that for each cultivar the influence of the rootstock on esca symptom appearance and on mortality rate was significant (Fig. 4). In general grafted vines did not display an evident tolerance to esca disease: grafted 'Sangiovese' and 'Chardonnay' vines showed a markedly higher percentage of symptomatic vines (81 % and 88 %, respectively) and mortality rate (77 % and 73 %, respectively) compared to the own rooted vines. Grafted 'Cabernet Sauvignon' and 'Trebbiano Toscano' vines also showed a high percentage of symptomatic vines (75 % and 56 %, respectively), however, in these cultivars mortality of the grafted vines was moderately low in comparison to the own rooted vines and did not exceed 34 %.

To determine the influence of rootstocks on the susceptibility of cultivars, the number of symptomatic, non-symptomatic and dead vines was considered (Fig. 5). The χ^2 test showed that both 1103P and K5BB significantly influenced esca symptoms appearance, but not mortality rate. Both rootstocks were associated with an increase in esca incidence in 'Sangiovese' and especially in 'Cabernet Sauvignon', of about 55 and 85 %, respectively. In the case of 'Chardonnay' the vines grafted on 1103P and K5BB showed a moderate increase in symptoms incidence that did not exceed 35 % and, as consequence, a high percentage of non-symptomatic vines (40 %) was found. Among the cultivars grafted only 'Trebbiano Toscano' showed a lower percentage of mortality and of symptomatic vines in comparison to own rooted vines. In fact, in Trebbiano Toscano there was a markedly low percentage of dead vines (< 6 %)

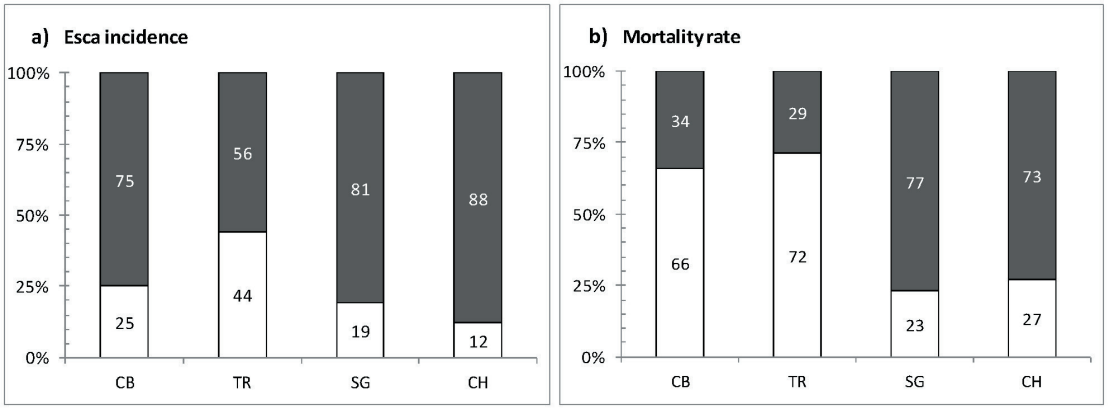

Fig. 4: Percentage of esca symptoms incidence (**a**) and mortality rate (**b**) in own rooted (□) and grafted (■) vines of 'Cabernet Sauvignon' (CB), 'Trebbiano Toscano' (TR), 'Sangiovese' (SG), 'Chardonnay' (CH).Values are calculated on the total number of symptomatic and dead vines recorded from 2004 to 2009. Differences in esca appearance between the cultivars are statistically significant ($\chi^2 = 11.04$, by $P < 0.025$). Differences in mortality rate between the cultivars are statistically significant ($\chi^2 = 8.95$, by $P < 0.05$).

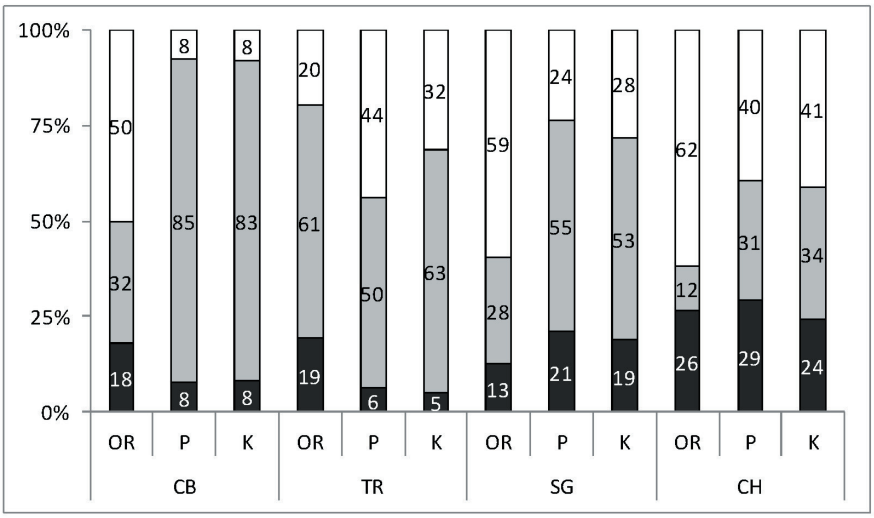

Fig. 5: Percentage of symptomatic (■) non symptomatic (□) and dead (■) vines own rooted (OR) and grafted on 1103 Paulsen (P) and Kober 5BB (K). Cultivars: 'Cabernet Sauvignon' (CB), 'Trebbiano Toscano' (TR), 'Sangiovese' (SG), 'Chardonnay' (CH). Values represent the situation at the end of 2009. Differences in numbers of symptomatic vines (own rooted and grafted) between rootstock combinations are significant for: $\chi^2 = 12.81$, $P < 0.005$ in CB; $\chi^2 = 8.78$, $P < 0.025$ in TR; $\chi^2 = 9.78$, $P < 0.010$ in SG; $\chi^2 = 6.37$, $P < 0.050$ in CH. Differences in numbers of dead vines (own rooted and grafted) between rootstock combinations are not significant for: $\chi^2 = 0.80$, $P < 0.5$ in CB; $\chi^2 = 4.01$, $P < 0.1$ in TR; $\chi^2 = 4.40$, $P < 0.1$ in SG; $\chi^2 = 1.05$, $P < 0.5$ in CH.

with both rootstocks and the percentage of non-symptomatic vines was high (44 % on 1103P and 32 % on K5BB) in comparison to own rooted vines (20 %) (Fig. 5).

Discussion

Climatic conditions, namely rainfall and temperature, are considered the main factors affecting the nature of foliar symptoms (Surico *et al.* 2000, Dubos *et al.* 2002, Marchi *et al.* 2006). In particular cool rainy summers favoured chronic esca, and hot dry summers favoured acute esca (Surico *et al.* 2000). At present, the precise role of rainfall in esca symptom expression has not been evaluated (Marchi *et al*. 2006). Due to the very high variability of symptom expression in our study we also were not able to find clear correlations between climatic conditions and esca expression. Similarly, soil characteristics were not correlated to the appearance of esca symptoms.

In this study the susceptibility of cultivars to esca appeared higher on grafted vines, and only 'Trebbiano Toscano' showed a similar percentage of symptomatic vines in both grafted and own rooted vines. Andolfi *et al.* (2011) report that symptoms of Petri disease (young esca) were more severe in grafted than in self-rooted cuttings. Borgo *et al.* (2008) observed that vigorous cultivars 'Sauvignon' and 'Dindarella', characterised by a higher trunk diameter, were more affected by esca disease compared to those with a lower development of the trunk. In our vineyard generally the own rooted vines were less vigorous than the grafted vines (*Scalabrelli G. personal communication*) and also showed lower incidence of esca. The own rooted vines avoid the phases of grafting during which aerial contamination by fungal spores can occur on weakened tissues caused by the grafting itself (Halleen *et al.* 2003, Hofstetter *et al*. 2012).

Despite the different characteristics of 1103P and K5BB rootstocks, i.e. 1103P more vigorous and drought resistant than K5BB, it was not possible to distinguish different influences of these rootstocks on the examined cultivars in relation to esca symptom expression. Similar results were reported by Materazzi *et al.* (1993) who found no significant difference in esca incidence among rootstock combinations of 'Sangiovese', 'Trebbiano Toscano' and 'Canaiolo nero' grafted on K5BB and 420A. A relationship between rootstocks and esca susceptibility was therefore excluded. The susceptibility of rootstock and grapevine cultivars to esca pathogens was also investigated by Eskalen *et al.* 2001, Marchi *et al*., 2001, Feliciano *et al*. 2004, and Billones-Baaijens *et al*. 2013, and these studies showed that, despite different levels of susceptibility to the pathogens observed among genotypes, none were truly resistant.

Marchi (2001) reported that 'Sangiovese' and 'Trebbiano Toscano' grafted on 1103P rootstock and own rooted, which are expected to be resistant to drought, showed unexpectedly high incidence of apoplectic stroke. Concerning the 1103P rootstock our results confirm this finding because 60 % of the total apoplectic stroke occurred on vines grafted on this rootstock. In contrast, this symptom was less frequent on vines grafted on K5BB (30 %) and on own rooted vines (10 %) (data not shown).

In relation to the high incidence of esca symptoms appearance, 'Cabernet Sauvignon' was confirmed as a genotype susceptible to esca disease, whereas 'Sangiovese' and especially 'Chardonnay' were found to be less susceptible, particularly when own rooted. 'Trebbiano toscano' appears to have intermediate susceptibility among the examined cultivars. Interestingly, the observed rates of mortality were not in accordance with the esca symptoms incidence. 'Cabernet Sauvignon' grafted showed a low percentage of dead vines, while 'Sangiovese' and 'Chardonnay' were

characterized by a very high mortality rate. These features are in accordance with Liminana *et al*. (2009), who showed that necrosis in grapevine wood was not always associated with foliar symptoms, but positively related with grapevine mortality. In our environmental conditions 'Sangiovese' and 'Chardonnay' could be infected by esca without showing the symptoms (hidden esca) for at least four years and only later might show esca symptoms. Based on the high mortality rate recorded during the last year of our survey we may infer that after long asymptomatic periods the most frequent symptom to appear may be apoplectic stroke. Due to the complexity of esca disease the evaluation of cultivar susceptibility is very difficult, particularly because it was not possible to know when infection started and several environmental factors appear to modify the appearance of symptoms. Due to this complexity we suggest that further experimental trials under field conditions for a minimum five-year period will be necessary to obtain an effective methodology for early detection.

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