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Incidence of grapevine trunk diseases on four cultivars in Sardinia, Southern Italy

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Summary

Esca proper and Botryosphaeria dieback are among the most widespread Grapevine trunk diseases (GTDs), characterized by similar decline symptoms. In the present work, chronic, apoplexy and death symptoms were analysed separately in four vineyards and four different cultivars, on more than 1,000 vines per cultivar, taking into account ten-year annual surveys. The cumulative incidence of plants with chronic symptoms (CHR) reached high values on 'Sauvignon Blanc' (81.9 %), 'Cabernet Sauvignon' (79.4 %) and 'Cannonau' (66.5 %), but it was low on 'Merlot' (25.1 %). 'Sauvignon Blanc' showed the highest cumulative incidence of apoplectic events (23.1 %) and dead cordons (49.2 %), while 'Cannonau' had the greatest number of dead plants (28.8 %). In each symptom category, incidence among cultivars differed significantly according to χ^2 test at $P \leq 0.05$. Annual incidence of foliar symptoms fluctuated over ten years (ranging from 0.9-9.5 % in 'Merlot' to 6.3-59.1 % in 'Cabernet Sauvignon'), mostly with regard to CHR. On average, every year only 33.9 % of plants showing CHR had expressed symptoms in the previous year, while 48.6 % did not show symptoms the following year. Conversely, most of the plants exhibiting apoplexy or death were symptomatic the previous year. According to Tuckey HSD test ($P \leq 0.05$) 'Merlot' had the highest incidence of plants showing CHR symptoms for the first time (72.1 %) and of apparently recovered plants (76.3 %), while 'Cabernet Sauvignon' exhibited the highest incidence of plants showing CHR symptoms also the previous year (50.0 %). The 'Cabernet Sauvignon' attitude to show chronic symptoms with a certain continuity was also confirmed by the low incidence of plants with hidden symptoms (lack of symptoms in previously symptomatic vines). On the contrary, the incidence of acute symptoms (apoplectic events and dead plants) was quite low on 'Cabernet Sauvignon'. The present study confirms that GTD incidence is influenced by cultivar. All the cultivars assessed were susceptible, but with differences in intensity, type (chronic or acute) and fluctuation of symptoms. It cannot be excluded, however, that besides the genotype also external factors, as the vigour conferred by the type of soil or the combination with the rootstock, may have influenced the results.

K e y w o r d s : Botryosphaeria dieback; 'Cabernet Sauvignon'; 'Cannonau'; esca proper; 'Merlot'; 'Sauvignon Blanc'.

Introduction

Grapevine trunk diseases (GTDs) are currently the main health problem affecting vineyards worldwide, as they attack the perennial organs of the plant leading to decrease in productivity and reduction in vine life span.

Esca disease is one of the major GTDs, which has been known for the longest time and has undergone several revisions. The latest revision considers esca to be a complex of two diseases: a tracheomycotic disease, named "grapevine leaf stripe disease" (GLSD; Surico 2009), associated with *Phaeomoniella chlamydospora* and several species of *Phaeoacremonium* (Larignon and Dubos 1997, Mugnai *et al*. 1999, Gramaje *et al*. 2015), and "esca" as a white wood rot (which gave the original name "esca" to the disease). The name "esca proper" is retained when the vascular syndrome, characterized by wood symptoms such as dark brown streaking (black spots in cross section) and brown-red necrosis around the pith, co-occurs with white rotted wood caused by basidiomycetes (*Fomitiporia mediterranea* being the most common species on grapevine in Europe; Fischer 2006). Foliar symptoms on established grapevines has been widely described (Mugnai *et al*. 1999, Surico *et al*. 2006, Lecomte *et al*. 2012, Bertsch *et al*. 2013) and include various types of discoloration, typically interveinal discolorations evolving into necrotic areas resembling tiger stripes. The symptomatic leaves can dry out and fall leaving defoliated and withered canes. This chronic syndrome contrasts with the severe or acute form in which asymptomatic plants can suddenly wilt within a few days. This acute event, named apoplexy, can result in partial or complete death of the vine.

Species in *Botryosphaeriaceae* have long been known as canker and dieback agents in different woody plants (Punithalingam and Holliday 1973, Punithalingam and Waller 1973), but their involvement in GTDs was underestimated or even ignored with few exceptions (Úrbez-Torres 2011 and references therein). Only in the 2000s, these fungi were recognized as among the most important grapevine pathogens because of their aggressiveness and wide geographic distribution (Phillips 2002, Úrbez-Torres 2011). About 40 species have been isolated from grapevines (Úrbez-Tor-

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res 2011, Linaldeddu *et al*. 2015, Pitt *et al*. 2015, Correia *et al*. 2016, Yang *et al*. 2017), most of which belonging to *Neofusicoccum* and *Lasiodiplodia* genera (Úrbez-Torres 2011), and each species can cause more than one symptom: bud mortality, shoot, spur, cane, cordon or trunk dieback, leaf chlorosis and anthocyanosis, leaf spots, fruit rot, bleached canes, cankers, apoplexy; wedge-shaped and arch-shaped necrotic lesion, brown-orange stripes just beneath the bark and black spots are present in the wood of cross-sectioned cordons and trunks (Larignon *et al*. 2001, van Niekerk *et al*. 2006, Úrbez-Torres 2011). Úrbez-Torres (2011) proposed the name Botryosphaeria dieback to describe the broad number of different symptoms caused by *Botryosphaeriaceae* species on grapevine.

Among the most widespread GTDs is also Eutypa dieback caused by the ascomycetous fungi *Eutypa lata*. It is characterized by stunted new shoots with small, deformed chlorotic leaves, canker and wedge-shaped wood necrosis (Moller *et al*. 1974, Bertsch *et al*. 2013).

Many aspects of GTDs still need to be clarified and these include the expression of external symptoms. It is worldwide known that plants showing foliar symptoms one year do not necessarily express them the following year (hidden symptoms). Knowing what factors limit symptom expression is important because diseased plants produce good yields when they are externally symptomless (Calzarano *et al*. 2004). Several assumptions have been made to explain this behaviour, for example vineyard management, cultivars and rootstocks, plant age and pedo-climatic conditions (Pollastro *et al*. 2000, Fussler *et al*. 2008, Di Marco and Osti 2009, Lecomte *et al*. 2011, Andreini *et al*. 2014, Kraus *et al*. 2019). Positive relationships between annual esca disease incidence and rainfall were found (Surico *et al*. 2000, Marchi *et al*. 2006, Guerin-Dubrana *et al*. 2013, Andreini *et al*. 2014, Calzarano *et al*. 2018, Serra *et al*. 2018, Kraus *et al*. 2019). Moreover, a strong correlation of GLSD symptom expression with temperature was recently shown by Calzarano *et al*. (2018) and Serra *et al*. (2018), with high temperature in summer hindering symptom expression.

Another topic refers to the possible attribution of external symptoms to the different GTDs. All major GTDs (Esca complex, Botryosphaeria and Eutypa diebacks) can cause apoplexy or bring the plant to death. As regards chronic symptoms, only Eutypa dieback differs clearly, while the esca complex and Botryosphaeria dieback have some features in common. For example, some authors believe that even Botryosphaeria dieback manifests tiger stripes similar to those of GLSD (Larignon *et al*. 2001), but there is no agreement on this (Lecomte *et al*. 2005, Úrbez-Torres 2011). It is indeed very difficult to assign particular symptoms to one disease because a variety of GTD fungi can be simultaneously isolated from adult vines (Luque *et al*. 2009, van Niekerk *et al*. 2011). The only way to definitively attribute one symptom to one pathogen is to test Koch's postulates, but it has proven difficult to reproduce foliar symptoms in inoculated plants. Recently, tiger stripes were reproduced in artificial inoculation of one-year old vines grown in pot with *Pa. chlamydospora* and different *Togninia/Phaeoacremonium* species (Úrbez-Torres *et al*. 2014), but also with *Neofusicoccum parvum* and *Diplodia seriata* (Reis *et al*. 2016). In the latter case, tiger stripes were reproduced on 30-70 % of the inoculated potted plants, depending on the isolate, and this result was replicated for 4 years.

Field observations and artificial inoculation trials indicated that diverse varieties and rootstocks had different levels of susceptibility to esca or Botryosphaeria dieback, but none were truly resistant (Marchi 2001, Eskalen *et al*. 2001, Borgo *et al*. 2008, Fussler *et al*. 2008, Landi *et al*. 2012, Bruez *et al*. 2013, Travadon *et al*. 2013, Andreini *et al*. 2014, Billones-Baaijens *et al*. 2014, Murolo and Romanazzi 2014, Guan *et al*. 2016, Sosnowski *et al*. 2016, Bellée *et al*. 2017, Ramirez *et al*. 2018). However, results obtained under laboratory conditions are sometimes inconsistent with field results; moreover, the susceptibility can be influenced by several factors such as geographic area, climate and cultivation technique. Since there are currently no effective control measures for preventing the occurrence of GTDs in both nurseries and vineyards, the identification of less susceptible varieties to these pathogens could provide new insights into the GTDs management.

Most of the above studies on different cultivar investigated the varietal susceptibility without distinguishing between chronic and acute symptoms and without assessing any differences in the fluctuation of symptoms. In addition, in Sardinia esca complex and Botryosphaeria dieback are the most widespread GTDs (Prota *et al*. 2002, Serra *et al*. 2010, Linaldeddu *et al*. 2010, 2015). Therefore, the objective of this study was to determine the field incidence of esca and Botryosphaeria dieback in four varieties commonly used in the Sardinia geographic area by a long-term monitoring survey (over 10 years), focusing on the expression of acute and chronic disease symptoms. Moreover, the four cultivars were analyzed in accordance with the symptom fluctuation in the ten years of survey.

Material and Methods

Site, vineyards and climate: Surveys were carried out in four vineyards (about 20 ha each) located in the Nurra wine region (north-western Sardinia) in the same farm (Tenute Sella & Mosca S.p.A., Alghero). Vine cultivars (three international and one local), rootstocks and year of planting are provided in Tab. 1. All vineyards were planted in north-south oriented rows in a plane area. Agronomic practices were the same in all vineyards: pergola-training, hand pruning, mechanical trimming, irrigation by under-head sprinklers, with exception of 'Merlot' that was drip irrigated, chemical weed management along the rows, manual or chemical desuckering, chemical fertilization, fungicide sprays against downy and powdery mildew

Table 1

Characteristics of the assessed vineyards

Cultivar	Rootstock	Year of planting
Sauvignon Blanc	SO4	1989
Cabernet Sauvignon	110R	1991
Cannonau	110R	1992
Merlot	1103P	1998

and mechanical harvesting. The trial area, located about 6 km from the sea, has a typical Mediterranean climate, i.e. mild and relatively rainy winters with dry and hot summers, remarkably windy. During the trial period (2002-2014) average annual rainfall was 607.1 mm of which 134.2 fell in May-September; average minimum temperature in the coldest months (January and February) was 3.7 °C, while average maximum temperature in the hottest months (July and August) was 31.1 °C (data obtained from the web site ARPAS, Sardinian Department of weather forecasting, http://www.sar.sardegna.it/, particularly from the weather station located at "Olmedo", which is about 4 km away from the trial site).

Disease assessment: In each vineyard, two blocks of 530 vines (10 rows x 53 positions, more than 1,000 vines in total) were randomly selected and symptoms assessed in late summer (from mid-August to early September, the period of major symptomatic expression). The survey was conducted from 2002 to 2011 for 'Sauvignon Blanc' (Saub), 'Cabernet Sauvignon' (Cabs) and 'Cannonau' (Can) and from 2005 to 2014 for 'Merlot' (Mer).

At the beginning of our survey, esca complex was the reference disease: tiger-stripe discoloration was the main symptom showed by monitored plants from whose wood *Pa. chlamydospora, Phaeoacremonium* spp. and *Fomitiporia* sp. were isolated. Simultaneously, also *N. parvum* and *D. seriata* were detected from the same plants. In light of the reproduction of tiger stripes in plants inoculated with *N. parvum* and *D. seriata* (Reis *et al.* 2016), we cannot rule out that also *Botryosphaeriaceae* species may have contributed to symptom occurrence in the assessed vineyard. For this reason, we prefer to use the term GTDs without distinguishing the two diseases. Chronic foliar symptoms (CHR) surveyed were: tiger-stripe discolorations, unspecific leaf chlorosis and necrosis (i.e. large areas of the leaf blade or edge of the leaf), and related leaf wilting, cane defoliation and wilt, cluster dehydration, bud mortality (Mugnai *et al*. 1999, Surico *et al*. 2006). Full sudden wilting (apoplexy, APO) and death (DEA, *i.e.* no sprouting in spring) of the whole plant or of one cordon were also assessed. The three symptom categories (CHR, APO and DEA) were processed separately.

Disease data were expressed as annual incidence, that is percentage of CHR, APO or DEA plants calculated on the total numbers of vines assessed each year. Cumulative incidence in each symptom category was considered as the percentage of all plants showing CHR, APO or DEA in at least one of the ten years of survey calculated on the total numbers of vines assessed in the first year. Regarding cumulative incidence, vines with only one dead cordon (DEA cordons) were evaluated apart from completely dead plants (DEA plants). As the four symptom categories (CHR, APO, DEA cordons and DEA plants) were processed separately, the same plant may have been considered in more than one category if it had manifested chronic symptoms, apoplexy or death in the same or different years.

To better define discontinuity in symptom expression the incidence of plants showing CHR in year n was divided into four subsets, modifying the procedure followed by Sosnowski *et al*. (2007) in their studies on Eutypa dieback:

1. NEW n: plants that showed symptoms for the first time in the year n;
2. SYM n - 1: plants that exhibited symptoms (CHR or APO) in year n and also in the previous year (n-1);
3. SYM n $\leq$ 2: plants that exhibited symptoms (CHR or APO) in year n and two or more years before (n $\leq$ 2) but were symptomless in the previous year;
4. ASY n + 1: vines that exhibited CHR symptoms in year n but were symptomless in the following year (n + 1).

Also vines with APO symptoms were divided into the same subsets as the CHR ones. Regarding DEA, the following subsets were considered: NEW n, that is a cordon or whole plant dead without showing any characteristic symptom before; SYM n - 1 and SYM n $\leq$ 2 as described above. The incidence of plants in every subset was calculated on the total number of plants in each symptom category (CHR, APO, DEA) in the year of assessment.

Statistical analysis: In all analyses the average disease incidences of the two blocks were considered. Since the cumulative incidence data were dichotomous (each plant could be symptomatic or asymptomatic), significant differences between cultivars in each symptom category (CHR, APO, DEA cordons and DEA plants) were assessed with the χ^2 test at $P \leq 0.05$. On the other hand, annual incidences in the various subsets (ten replicates corresponding to the annual incidence in the ten years of survey), were arcsin-square root transformed prior to be analysed as a function of cultivars and subsets by two-way ANOVA. Differences among cultivars within subsets and among subsets within cultivars were analyzed by one-way ANOVA. Means were separated using the Tukey HDS test at $P \leq 0.05$.

Results

Annual incidence of symptomatic plants differed among the four cultivars and showed the usual fluctuations each year. As illustrated in Figure 1 manifest symptoms (CHR + APO) increased or decreased every year. However, if we considered every plant that was symptomatic in at least one of the previous years (hidden symptoms) and dead plants, the incidence rose year after year reaching very high levels except on Mer. Considering only the most affected cultivars, Cabs often shows the lowest incidence of hidden symptoms.

Concerning the cumulative incidence of plants showing CHR, APO, DEA cordons and DEA plants in the ten years of survey (Tab. 2), Saub showed the highest values in all categories except DEA plants, while Mer showed the lowest incidences. The local cv Can showed the highest incidence of dead plants. In each symptom category, incidence among cultivars differed significantly. Comparing cultivars in pairs, differences were significant in all symptom categories except CHR symptoms between Saub and Cabs ($\chi^2 = 2.23$, $P > 0.1$).

Differences in disease incidences among cultivars affected disease incidence among subsets. To exclude this influence, we considered subset incidences calculated on the total number of plants in each symptom category. A clear trend in symptom evolution was identified only in symptom categories of cultivars where incidence was higher.

Table 2

Cumulated incidences (%), *i.e.* incidence of plants showing chronic, apoplexy or death symptoms in at least one of the ten years of survey, in cultivars 'Sauvignon Blanc' (Saub), 'Cabernet Sauvignon' (Cabs), 'Cannonau' (Can) and 'Merlot' (Mer)

Cultivar	Chronic[a]	Apoplexy[a]	Dead cordons[a]	Dead plants[a]
Saub	81.9	23.1	49.2	16.6
Cabs	79.4	8.6	32.0	6.8
Can	66.5	17.9	41.4	28.8
Mer	25.1	1.6	1.0	2.9

[a] Significant differences among cultivars for chronic ($\chi^2 = 936.06$, $P < 0.001$), apoplexy ($\chi^2 = 259.29$, $P < 0.001$), dead cordons ($\chi^2 = 660.75$, $P < 0.001$) and dead plants ($\chi^2 = 351.82$, $P < 0.001$).

As expected, the incidence of plants that showed symptoms for the first time (NEW subset) constantly and considerably declined in the ten years of the survey. Also, the number of plants that apparently recovered the year after the appearance of CHR (ASY n + 1 subset) decreased, though irregularly. On the contrary, the incidence of plants that showed symptoms also in previous years (SYM n - 1 and SYM n ≤ 2 subsets) tended to increase but in a very irregular way. Rather different trends were observed in symptom categories of cultivars with low cumulative disease incidence, particularly APO and DEA of Cabs and all categories of Mer, in which incidence in all subsets fluctuated without any definite trend.

Analysis of variance for cultivars and subset on the annual incidence of plants showing CHR, APO or DEA showed significant F ratio ($P \leq 0.05$) for the subset factor in all the symptom categories (Tab. 3). CHR incidence in NEW n and ASY n + 1 subsets were significantly higher than incidence in SYM n - 1 and SYM n ≤ 2 subsets (Tab. 4, "Subs" section). The majority of APO (cordon or whole plant) had shown some kind of symptoms in the same or in the previous year (subset SYM n-1) while a small number only were symptomless the following year (subset ASY n + 1); likewise, most DEA (cordon or whole plant) showed CHR or APO the year before.

In contrast, F ratio was not significant for the cultivar factor (Tabs 3 and 4 "Cv" section), but significant cultivar x subset interaction was observed in the symptom category CHR and DEA. For this reason, cultivar data were analysed subset by subset and subset data were analyzed cultivar by cultivar. Significant differences among cultivars appeared within subsets, most of all in the CHR category (Fig. 2): Mer showed the highest number of plants in the NEW n and ASY n + 1 subsets but the lowest in SYM n - 1 subset, while Cabs showed the highest number of plants in the SYM n - 1 subset. As regards DEA category, significant differences among cultivars were detected only in the NEW n subset, in which the number of Can vines or cordon died without exhibiting any symptoms before was significantly different from Saub. Differences among subsets within cultivars reflected what was already described for the mean values

Table 3

Analysis of variance for cultivar and subset on the incidence of plants showing chronic, apoplexy or death (cordon or whole plant) symptoms. F ratio is significant at $P \leq 0.05$

Source of variation	Chronic				Apoplexy				Death			
	DF	Mean square	F ratio	*P* value	DF	Mean square	F ratio	*P* value	DF	Mean square	F ratio	*P* value
Cultivar	3	201.79	1.64	0.184	3	187.64	0.51	0.674	3	26.32	0.19	0.906
Subset	3	2143.74	17.38	0.000	3	14043.52	38.39	0.000	2	9625.46	67.67	0.000
Cultivar x subset	9	1362.69	11.05	0.000	9	644.99	1.76	0.083	6	412.24	2.9	0.012
Residual	124	123.33			117	365.81			92	142.26		
Total (corrected)	139				132				103			

Table 4

Ten years average incidences of plants showing chronic (CHR), apoplexy (APO) or death (cordon or whole plant, DEA) symptoms in four cultivars (Cv) and four subsets (Subs)

Cv[a]	CHR	APO	DEA	Subs[b]	CHR	APO	DEA
Saub	34.9 a[c]	27.7 a	32.7 a	NEW n	45.8 a	22.8 b	25.3 b
Cabs	34.1 a	26.8 a	33.7 a	SYM n-1	33.9 b	63.8 a	60.6 a
Can	37.4 a	26.5 a	33.6 a	SYM n ≤ 2	22.4 b	15.4 bc	14.5 c
Mer	44.3 a	25.6 a	33.9 a	ASY n+1	48.6 a	4.5 c	

[a] Saub: 'Sauvignon Blanc'; Cabs: 'Cabernet Sauvignon'; Can: 'Cannonau'; Mer: 'Merlot'.
[b] NEW n: plants that showed symptoms for the first time in year n; SYM n-1: plants that showed symptoms in year n and also in the previous or in the same year; SYM n ≤ 2: plants that exhibited symptoms in year n and two or more years before but were symptomless in the previous year; ASY n+1: vines that exhibited symptoms in year n but were symptomless in the following year.
[c] Values followed by the same letter along the column do not differ statistically according to Tuckey HSD test ($P \leq 0.05$).

except for CHR category: Saub and Can did not show any significant differences among subsets, while the incidence of Cabs vines in SYM n - 1 subset was significantly higher than that of plants in SYM n ≤ 2 subset.

Discussion

The incidence of symptomatic plants, both annual and cumulative, reached very high levels in all cultivars except for Mer. This result might be correlated with the age of Mer vineyard as it is known that the incidence of symptomatic plants is higher in old vineyards (Surico *et al*. 2006, Kraus *et al*. 2019), although the age difference is not so high to justify alone the significant difference in incidence of symptomatic vines (Fig. 1, Tab. 2). Indeed, the low field susceptibility of Mer to both the esca complex and the Botryosphaeria dieback, especially compared to Cabs, has already been reported (Christen *et al*. 2007, Borgo *et al*. 2008, Fussler *et al*. 2008, Quaglia *et al*. 2009, Bruez *et al*. 2013, Murolo and Romanazzi 2014, Sosnowski *et al*. 2016, Guerin-Dubrana *et al*. 2019). It is likely that this tolerance is due to genetic traits since Lambert *et al*. (2013) demonstrated that Mer showed an earlier and stronger defense response than the susceptible Cabs when treated with *Pa. chlamydospora* cultural filtrates. Furthermore, Pouzoulet *et al*. (2014, 2017) found that Mer plants with small diameter vessels might be able to occlude them in a quicker and more efficient manner than plants with large diameter vessels like Cabs, thus restricting movement of fungi and toxins. Saub was also shown to be highly susceptible to both diseases (Borgo *et al*. 2008, Fussler *et al*. 2008, Bruez *et al*. 2013, Murolo and Romanazzi 2014, Sosnowski *et al*. 2016), while no report exists on Can susceptibility, the main cultivar planted in Sardinia. However, this variety is genetically identical to other Italian, Spanish and French cultivars, among which the best known is Grenache (Meneghetti *et al*. 2011, Mercenaro *et al*. 2016), which, in the French National Grapevine Wood Disease Survey (Fussler *et al*. 2008) and in a survey conducted in Australia (Sosnowski *et al*. 2016), was placed in an intermediate position between the most resistant and the most susceptible cultivars. Also, Grenache was among the least affected by GTDs in a European survey (Guerin-Dubrana *et al*. 2019).

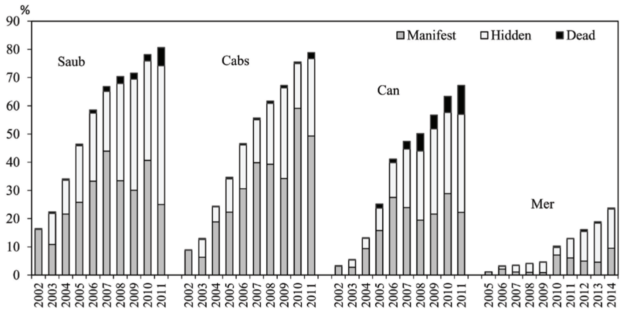

Fig. 1: Frequency of dead plants and of plants with manifest symptoms (chronic and apoplectic) and hidden symptoms (symptomless in the year of survey but symptomatic, *i.e.* chronic and/or apoplectic, in previous years) on 'Sauvignon Blanc' (Saub), 'Cabernet Sauvignon' (Cabs), 'Cannonau' (Can) and 'Merlot' (Mer) in the ten years of survey

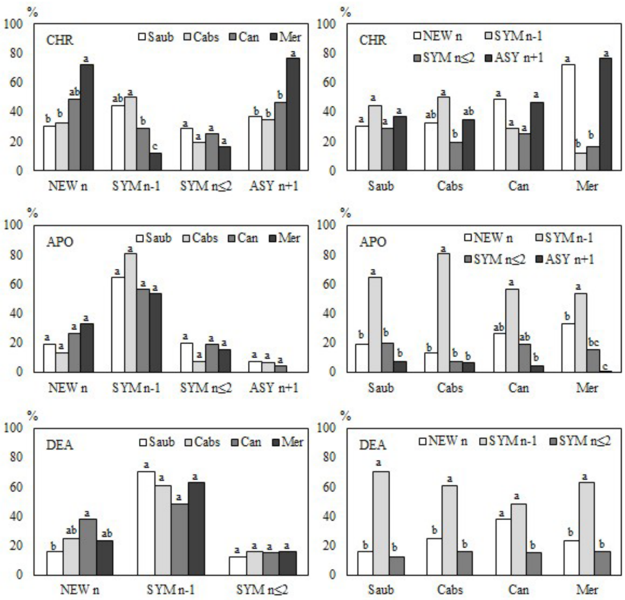

Fig. 2: Ten years average incidences of plants showing chronic (CHR), apoplexy (APO) or death (cordon or whole plant, DEA) symptoms in four cultivars (see Tab. 4), analysed subset by subset (graphs on the left), and four subsets (see Tab. 4), analyzed cultivar by cultivar (graphs on the right). Values followed by the same letter in each group of histograms do not differ statistically according to Tuckey HSD test ($P \leq 0.05$).

In the present study both Saub and Cabs showed high and similar cumulative incidence of plants with chronic symptoms, but the incidence of acute symptoms, most of all apoplectic events and dead plants, was quite low in Cabs, the nearest to that of Mer albeit significantly different (Tab. 2). The incidence of Can plants in the different symptom category was intermediate, except in the dead plant category where a very high value was reached.

In addition to the genetic and anatomical features described above, there may be other reasons to explain differences in symptom expression. Even if situated in the same farm and subjected to the same cultural practices the four 20 ha vineyards were located in a 600-ha plain area, where several soil types could create different growth conditions. In this regard, it was found that soils with high percentage of loam and clay and with either high water reserve or high nitrogen supply were more conducive to symptom expression, indicating a link between esca and plant vigor (Goutouly 2007, Lecomte *et al*. 2011). Moreover, also the rootstock can contribute to the vigor of the cultivar and Cabs and Can had a different rootstock from that of Saub (Tab. 1). A vigorous plant requires a greater amount of water and nutrients and can more easily undergo stress conditions, for example a dysfunction of the xylem apparatus or the circulation of toxins. Indeed, Saub and Can vines were visually more vigorous than Cabs plants and this could explain the lower number of apoplectic and dead plants of the latter (Tab. 2). As described before, however, there may be genetic causes currently unknown. Since Can is very susceptible to downy mildew, the high number of dead plants recorded in 2008 is probably due to the unusual downy mildew epidemic occurring in 2007, which greatly restricted vegetative growth and so hindered nutrient accumulation in canes necessary to support early growth in the following spring (Keller 2015). Consequently, in 2008 bud burst was stunted and many vines did not sprout at all. The assumption that external causes may have triggered the plant death would seem corroborated by the highest percentage of Can plants dead without ever having shown GTDs symptoms. However, due to anomalies in the onset of symptoms typical of GTDs, it seems more realistic to assume that downy mildew epidemic may have acted synergistically to GTDs in inducing the death of many plants. Moreover, the tendency to death of Can GTDs affected plants is a common trait in Sardinian vineyards, therefore it could be determined by cultivar features. For example, 'Grenache' is considered vulnerable to cavitation (Keller 2015) and this event, amplified by water stress conditions and exacerbated by the dysfunction of infected wood, could weaken the plant to death.

As repeatedly reported throughout world's vine-growing areas, and as seen in this trial, the annual incidence of foliar symptoms fluctuated in the ten years of survey, and this was most evident in the chronic symptoms. On average for all cultivars, each year only 33.9 % of CHR plants had expressed symptoms the year before, while 48.6 % did not show symptoms in the following year (Tab. 4). This could be also an indirect indication of the large disproportion between internal vs. external symptoms reported by other authors (Serra *et al*. 1998, Calzarano *et al*. 2007). On the contrary, most of the plants exhibiting apoplexy or death were symptomatic the year before, confirming previous studies (Stefanini *et al*. 2000, Péros *et al*. 2008, Guerin-Dubrana *et al.* 2013, Kraus *et al.* 2019).

The high incidence of Mer plants that showed CHR symptom for the first time and of apparently recovered plants (subsets NEW n and ASY n + 1, Fig. 2) compared to that of plants that showed symptoms in previous years (SYM n - 1 and SYM n ≤ 2 subsets) was consistent with the low cumulative disease incidence in this cultivar. Conversely, in the varieties with high cumulative incidence differences among subsets flatten out except for Cabs. Interestingly, the high incidence of plants that showed symptoms also the year before (SYM n - 1 subset) indicate the Cabs attitude to show chronic symptoms with a certain continuity. This is also confirmed by the low annual incidence of plants with hidden symptoms (Fig. 1) and is in accordance with the results of Andreini *et al*. 2014.

Symptom fluctuation was smaller in APO category: 63.8 % of APO cordons and plants were symptomatic the year before, while only 4.5 % were symptomless the year after. The latter had suffered apoplexy at the end of August, when canes had already accumulated nutritional reserves to support early vegetative growth the next season. On the other end, few apoplectic plants (usually under 1 % of the annually assessed plants) appeared very early, before the end of May. In most cases these were plants that had stopped their growth from bud burst to inflorescences appearance, probably due to the lack of nutritional reserve for GTDs symptoms in the previous season. A fair percentage of vines had undergone apoplexy without having previously shown GTD symptoms (NEW n subset). Although there were no statistical differences between cultivars, the lowest incidence was recorded on Cabs, the highest on Mer (Fig. 2). In the first case this confirms the continuity in appearance of symptoms already underlined for Cabs. In Mer, considering the overall low cumulative disease incidence, we cannot exclude the involvement of causes other than Botryosphaeria dieback or esca complex. For example, eutypiose symptoms were sporadically assessed, although vines exclusively showing the characteristic foliar symptoms were excluded from the data set.

The same considerations can be made for the DEA category; indeed, the death of a cordon or a plant can be triggered by many causes, not all of them with a parasitic background. Anyway, the incidence of vines dead without showing symptoms before (subset NEW n) was significantly lower than that of previously symptomatic vines in all cultivars except Can (Fig. 2).

The present study indicates that GTD incidence may be influenced by the cultivar. All the cultivars assessed in our study were susceptible, but with differences in intensity, type (chronic or acute) and fluctuation of symptoms. As already mentioned, age and genotype can affect the susceptibility of the cultivar, but it cannot be excluded that also external factors, as the vigor conferred by the type of soil or the combination with the rootstock, may have influenced the results. In any case, the choice of cultivar cannot depend exclusively on susceptibility to GTDs, especially in Mediterranean countries where wine production is closely linked to the territory. The Can grapes are used to produce the appellation of origin DOC 'Cannonau' of Sardinia, which represents the most profitable production for Sardinian winemakers and cannot be replaced by another cultivar. Genetic adjustment is possible, as was demonstrated by the recent introduction of introgression lines resistant to downy and powdery mildew (Töpfer *et al*. 2011, Pertot *et al*. 2017), but it is still an open question with new cultivars that need proper wine and commercial exploitation. At present, the best approach to limit symptom appearance of GTDs is integrated management based on good quality of propagation material and adequate cultivation techniques (Gramaje *et al*. 2018).

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