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RESEARCH PAPERS - 10TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Expression of grapevine leaf stripe disease foliar symptoms in four cultivars in relation to grapevine phenology and climatic conditions

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Summary. Grapevine leaf stripe disease (GLSD) symptom expression was analysed in four vineyards and four cultivars, in Sardinia (Italy), taking into account ten-year annual and five-year monthly surveys. The cumulative incidence of symptomatic plants reached high values on Sauvignon blanc, Cabernet sauvignon and Cannonau (81.9, 79.4 and 66.5% respectively), but low on Merlot (25.1%). Symptoms appeared during or before the 50% flowering stage and maximum increments were assessed in June and partially in July. Annual incidence of foliar symptoms fluctuated in the ten years of the survey. Positive regressions were found between incidence of vines that exhibited foliar symptoms in year n but were symptomless in year n-1 and rain parameters in the 30 days after stabilization of mean temperature around 10°C, when colonization of pruning wounds begins. This relationship could suggest the involvement of new infections or re-infections on symptom expression in the following growth season. Significant regressions between incidence of vines that exhibited foliar symptoms in year n but were symptomless in the year n+1 and climatic parameters were also recorded. High temperatures and low rainfall in the period from pre-flowering to veraìson were conducive to a higher number of asymptomatic plants. Regarding monthly foliar symptoms evolution, an increase in temperature from 50% sprouting until June led to a greater number of new symptomatic plants. On the other hand, a smaller percentage of new symptomatic plants was associated with an increase in temperature from June to July, which may have influenced vine water balance and transport of toxins by the sap flow.

Key words: vineyards, disease progress, rainfall, temperature.

Introduction

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

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 (Surico, 2009): a tracheomycotic disease, named "grapevine leaf stripe disease" (GLSD), associated with *Phaeomoniella chlamydospora* and several species of *Phaeoacremonium* (Larignon and Dubos, 1987; 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-occur with white rotted wood caused by basidiomycetes, of which *Fomitiporia mediterranea* is the most common species on grapevine in Europe (Fischer, 2006). Other wood pathogens, mainly canker agents, can colonize the same plants and the damage they cause can intermingle with the vascular and decay symptoms (Bertsch *et al*., 2013). Foliar symptoms on established grapevines have 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,

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typically interveinal discolorations evolving into necrotic areas resembling tiger stripes. The symptomatic leaves can dry out and fall leaving defoliated and withered canes. This syndrome contrasts with the apoplectic form in which asymptomatic plants suddenly wilt within a few days. Apoplexy can result in partial or complete death of the vine.

Since the fungi are confined to the wood, one of the most probable cause for foliar symptom expression is the xylem transport of toxic substances produced in the wood by pathogenic fungi that trigger a plant response (Andolfi *et al*., 2011; Calzarano *et al*., 2016, 2017a, 2017b), more than the failure to supply water and nutrients caused by impairment of the vascular system. This view is supported by recent studies at molecular level on alteration of plant metabolism caused by the main esca complex fungi (for review see Bertsch *et al*., 2013 and Fontaine *et al*., 2016).

Discontinuity in foliar symptom expression is another unresolved topic of GTDs. It is known worldwide that plants showing GLSD foliar symptoms one year do not necessarily express them the following year. Knowing what factors limit symptom expression is important because diseased plants produce good yields when they are symptomless (Calzarano *et al*., 2004). Consequently, measures aimed at reducing leaf symptom expression can also reduce yield losses (Calzarano *et al*., 2014). Nevertheless, the reasons for this fluctuation have not been fully established but several hypotheses have been advanced. A number of factors could influence symptom expression, for example vineyard management, pruning and trellising systems, 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).

However, the main factor that changes from one year to the next is climate, particularly rainfall and temperature. Positive relationships between annual esca disease incidence and rainfall in the June–August period are usually found (Surico *et al*., 2000; Guerin-Dubrana *et al*., 2013; Andreini *et al*., 2014) with some exceptions. Similarly, Marchi *et al*. (2006) found that rainfall between May–July was inversely related to hidden esca (lack of symptoms in previously symptomatic vines) in two out of three vineyards. The common hypothesis is that rain increases availability of water in the soil and consequently increases sap flow in the xylem. This flow indirectly influences the movement of toxins up to the leaves, where they cause symptoms. Indeed, leaf transpiration is the main driving force for water uptake and it increases linearly with increasing temperature, but closure of stomata prevents excessive loss of water by reacting to various environmental variables, such as water stress, a decrease in relative humidity or very high temperatures (Keller, 2015). Therefore, temperature can also influence sap flow and toxin transportation. While relationships with rain have been investigated quite extensively, the influence of temperature has barely been studied and no relation has been found (Andreini *et al*., 2014), although recent studies have been undertaken in this context (Calzarano F. *et al*., 2018).

With the aim of contributing to an advance in our understanding on the role of climate on esca complex and to clarify foliar symptom discontinuity, we analysed GLSD symptom expression in four vineyards of different varieties, located in the same farm, taking into account ten-year annual and five-year monthly surveys. Disease incidence was associated with rainfall and temperature data at different periods and in relation to grapevine phenology. In particular in this paper we tested 2 hypothesis:

1) rain and warm temperatures before flowering may influence the appearance of symptoms as they favor vegetative growth and therefore transport of toxic metabolites from wood to leaves, causing increased symptom expression

2) the effect of rain after flowering should be the same, but high summer temperature can hinder transpiration and sap flow, which may result in lower symptom expression.

Materials and methods

Site, vineyards and climate

Surveys were carried out in four vineyards (about 20 ha each) located in the Nurra wine region (northwestern Sardinia, Italy) in the same farm (Tenute Sella & Mosca S.p.A., Alghero). Vine cultivars (three international and one local), rootstocks and age are provided in Table 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

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

fertilization, fungicide sprays against downy and powdery mildew and mechanical harvesting.

The trial area is about 6 km from the sea and has a typical Mediterranean climate, i.e. mild and relatively rainy winters with dry and hot summers, and remarkably windy. In the 30-year period 1971–2000 (data obtained from the Air Force weather station located at the Alghero-Fertilia Airport, which is about 4 km from the monitored vineyards, http://clima.meteoam.it/atlanteClimatico.php) average annual rainfall was 573.4 mm of which 112.3 fell in May–September. Average minimum temperature in the coldest months (January and February) was 4.3 °C, while average maximum temperature in the hottest months (July and August) was 29.6 °C.

Daily cardinal temperatures (maximum, minimum and average) and daily rainfall in the assessment years were 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. Tenute Sella & Mosca provided climatic and phenological data for the four cultivars.

Disease assessment

In each vineyard two blocks of 530 vines (10 rows x 53 positions, more than 1000 vines in total) were randomly selected and symptoms assessed in late summer (from the end of August to early September, the period of major symptomatic expression in Sardinia) from 2002 to 2011 for Sauvignon blanc, Cabernet sauvignon and Cannonau and from 2005 to 2014 for Merlot. From 2005 to 2009 for Sauvignon blanc, Cabernet sauvignon and Cannonau and from 2010 to 2014 for Merlot the assessments were carried out monthly from the end of May, when first symptoms appear, to the end of August or early September. GLSD symptoms surveyed were: tiger-stripe discolorations, and related leaf wilting, cane defoliation and wilt, cluster dehydration, bud mortality.

Disease data were expressed as monthly or annual incidence that is percentage of GLSD symptomatic plants calculated on the total numbers of vines assessed each year (which varies according to the number of plants still standing in each year). Cumulative incidence was considered as the percentage of all plants showing GLSD in at least one of the ten years of survey calculated on the total number of vines assessed in the first year.

To define discontinuity in symptom expression the incidence of plants showing GLSD in year n was divided into two subsets, following the method used by Sosnowski *et al*. (2007) in their studies on Eutypa dieback:

1. Asymptomatic in year n-1 (ASY n-1): vines that exhibited GLSD in year n but were symptomless in the previous year;
2. Asymptomatic in year n+1 (ASY n+1): vines that exhibited GLSD in year n but were symptomless in the following year.

As explained in the next section, this approach would indicate which are the climatic factors determining the appearance of symptoms in a vine that was asymptomatic the year before or the disappearance of symptoms in a vine that was symptomatic in year n. The incidence of asymptomatic vines in year n-1 (ASY n-1 subset) was calculated on the total number of vines assessed each year; the incidence of asymptomatic vines in year n+1 (ASY n+1 subset) was calculated on the total number of plants showing symptoms in year n.

Relationship between disease incidence and climatic factors

Attempts were made to associate disease incidence and climatic factors in different combinations also according to grapevine phenology. Firstly, GLSD incidences were separately related with rain and temperature parameters during different periods. The first period covers all climate events from the beginning of the vegetative season (50% sprouting) to the final assessment; this period was split into two parts, before and after flowering.

1. First part of the growth season, from 50% sprouting to 50% flowering;

2. second part of the growth season, from 50% flowering to the last assessment.

Climatic parameters tested over the above periods were: total rainfall and rainfall per day in millimeters, total number of rainy days, number of days with more than 1, 5, 10, or 15 mm of rain, average cardinal temperatures and numbers of days with temperatures below and above different values depending on the investigated period (Table 2).

The influence of the climate on the discontinuity in the appearance of GLSD was then investigated.

1) Symptom development (ASY n-1): to explore the factors leading an asymptomatic vine to show symptoms in the following year (new symptomatic vines), the hypothesis tested was that mild temperature and rain during winter or early spring promote production and spread of fungal inoculum and therefore new infections on healthy or already infected plants. Two periods were considered: February–March, and 30 days after stabilization of average temperature around 10°C, when vascular fungi begin the colonization of pruning wounds (Serra *et al*., 2008). Rain and temperature parameters tested were the same as described for annual incidence.

2) Symptom disappearance (ASY n+1): to explore the factors leading a symptomatic vine to become asymptomatic in the following year (vines with hidden symptoms), two hypothesis were analysed. The first considered pathogen mortality or impairment caused by very low temperature during winter before the symptomless growth season. For this purpose, incidence of ASY n+1 plant was associated with cardinal temperatures and with the number of days with temperatures below different values during January–February (Table 2). The second hypothesis considers the influence of temperature and rain on sap flow in the year n+1 as explained above. Then, ASY n+1 incidence was related with the same rain and temperature parameters described for annual incidence but only in three periods: March–April, May–June and June–July.

Table 2. Minimum (Min), average (Med) and maximum (Max) temperatures (°C) considered to obtain number of days below (<) or above (>) the indicated value in different periods.

Period	Min	Med	Max
50% sprouting - last assessment	–	<12, >26	–
50% sprouting - 50% flowering	<5	<12, >20	>27
50% flowering - last assessment	–	>25, >27	>33, >35
January - February	<0, <2	<5, <8	–
February - March	>8, >10	>12, >14	–
March - April	>10	>15	>20
May - June	–	>23, >25	>30, >33
June - July	–	>25, >27	>33, >35
30 days after stabilization of average temperature around 10°C	>8, >10	>10, >12	

It is important to point out that Sosnowski *et al*. (2007) considered the same subsets to study discontinuity in Eutypa symptoms appearance, but given the difference between GLSD and Eutypa dieback they tested different hypothesis.

Regarding monthly GLSD, the incidences of new symptomatic plants in each of the four assessments (in the first all symptomatic plants were considered) were related with climatic parameters between two consecutive assessments. Before the first assessment climatic data since 50% sprouting was considered. To highlight any critical periods for the appearance of symptoms during the vegetative season, the incidence of new symptomatic plants in the first and second assessment was related with the climatic parameters from 50% sprouting to the first assessments and from the first to the second assessment. Similarly, the incidence of new symptomatic plants in the second and third assessments, and that in the third and fourth assessments was related with climatic data in the same periods. Climatic parameters considered were rainfall, rainfall per day and average cardinal temperatures. As described above the hypotheses tested were: more rain in spring and in summer and warmer temperature in spring would lead to more symptomatic plants; hotter temperatures in summer would result in fewer symptomatic plants.

Statistical analysis

In all analyses the average disease incidences of the two blocks were considered. Relationships between climatic factors (independent variables) and disease incidences (dependent variables) in different combinations (see previous section) were evaluated by regression analysis separately for each cultivar. We chose to test different models and several climatic parameters (Sosnowski *et al.*, 2007) to avoid losing important relationships that would not be highlighted with just one of them.

Four regression models were considered:
Linear, $y = a + bx$;
Logarithmic, $y = a + b \ln(x)$;
Quadratic, $y = a + bx + cx^2$;
Exponential, $y = a e^{bx}$;
where "y" is the dependent variable (arcsin-square root transformed disease incidence), "x" the independent variable (climatic factor), "a" the constant term, "b" and "c" regression coefficients. Statistical significance of the models was checked by an F-test of the overall fit ($P \leq 0.01$), followed by t-tests of individual parameters (a, b and c, $P \leq 0.05$) and by Shapiro-Wilk-test of residuals ($P > 0.05$). Goodness of fit to the models was evaluated by the coefficient of determination (R^2). Only R^2 values ≥ 0.7 were considered. All statistical analyses were performed with IBM SPSS Statistic version 20.

Results

Disease incidence

The cumulative incidence of plants showing GLSD in the ten years of survey reached very high levels on Sauvignon blanc (81.9%), Cabernet sauvignon (79.4%) and Cannonau (66.5%), but low on Merlot (25.1%). In addition, the annual incidence of symptomatic plants differed among the four cultivars and showed the usual fluctuations each year (Figure 1). On average, each year 68 % of symptomatic plants had been symptomless the year before, while 48.6% did not show symptoms in the following year. Fluctuations between years were similar in the different cultivars with two exceptions. Thus, in 2007 incidence rose in Sauvignon blanc and Cabernet sauvignon but declined in Cannonau and Merlot, while in 2009 incidence increased in Cannonau and decreased in all the others cultivars.

As regards monthly symptom evolution, first GLSD symptoms appeared before the end of May on a few plants of Sauvignon blanc, Cabernet sauvignon and Cannonau. In all these cultivars the greatest increase was recorded in June, with a small increase in August, except for 2006. First symptoms became visible before the end of May also on cv. Merlot, but the greatest increase was recorded in June only in 2011 and in July from 2012 to 2014. In 2010 foliar symptom incidence increased regularly.

Relationship between disease incidences and climatic factors

The trend of temperature and rainfall throughout the experimental period is shown in Figure 2. In this paper, we report only the relationships significant for more than one of the examined cultivars.

In general, regardless of the disease incidence considered, goodness of fit to the tested models was different among periods, climatic parameters or cultivars. Nevertheless, except where indicated, the trend of the significant regressions was the same for all the rain or temperature parameters in the different cultivars.

Annual disease incidence

No significant relationship with $R^2 \geq 0.7$ among GLSD annual incidences and the climatic factors tested from 50% sprouting to the final assessment or to flowering was found. From flowering to the final assessment, no significant relation was valid for more than one cultivar.

Symptom development

Concerning the incidence of vines that exhibited foliar symptoms in year n but were symptomless in year n-1 (ASY n-1 subset) several significant regres-

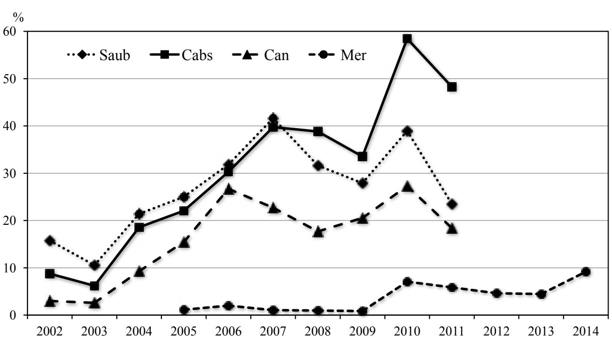

Figure 1. Annual incidence of plants with GLSD foliar symptoms on Sauvignon blanc (Saub), Cabernet sauvignon (Cabs), Cannonau (Can) and Merlot (Mer) in the ten years of survey.

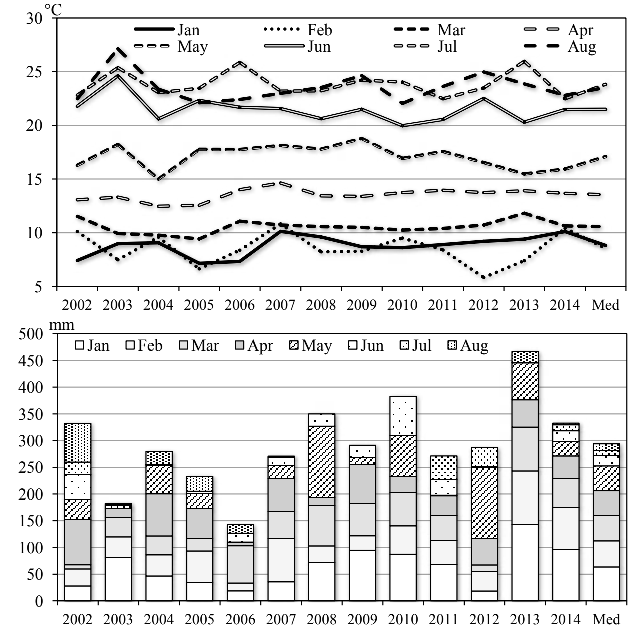

Figure 2. Monthly average temperatures (°C, lines) and monthly total rainfall (mm, bars) measured near assessed vineyards in the trial period. Med represent the average values from 2002 to 2014.

sions with rain parameters were found in the 30 days after stabilization of average temperature around 10°C. In particular, symptoms in all cultivars, except Merlot, were significantly related with the number of days with more than 5 mm of rain. Linear and exponential regression models gave good fit to these relationships, but with linear regression showing the best R^2. The linear regressions showed that the incidence of symptomatic plants that were symptomless the previous year increased as the number of days with more than 5 mm of rain increased (Figure 3). No significant regression with temperature parameters valid for more than one cultivar was found.

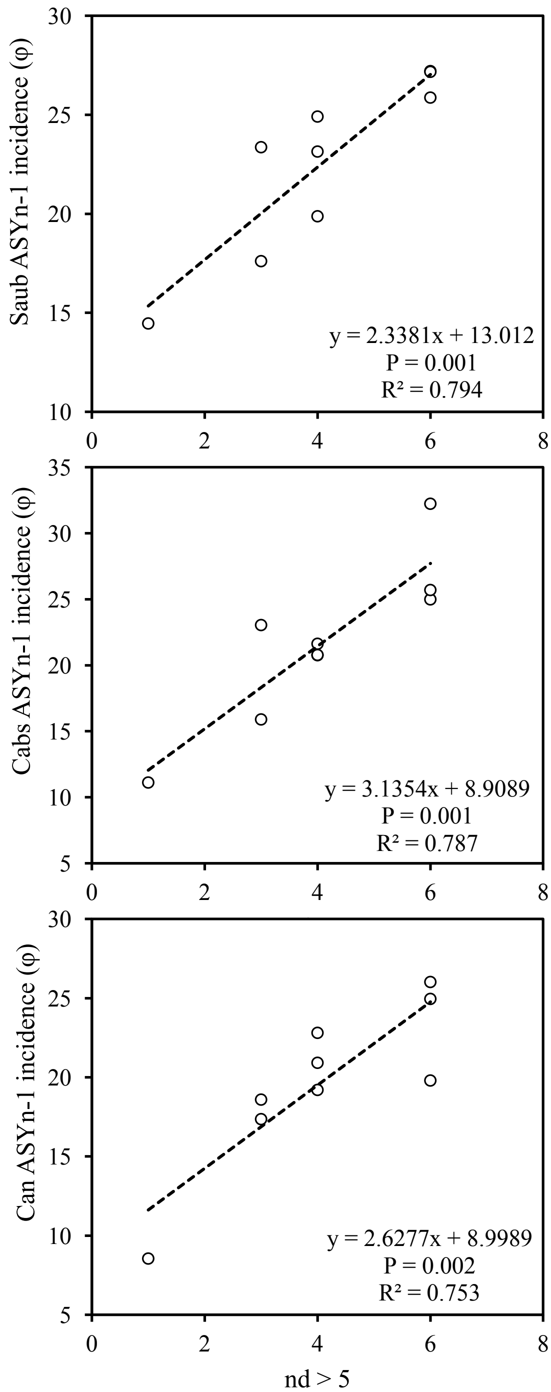

Figure 3. Regressions between arcsin-square root transformed (degrees φ) annual incidence of Sauvignon blanc (Saub), Cabernet sauvignon (Cabs) and Cannonau (Can) plants showing GLSD foliar symptoms in year n but symptomless in year n-1 (ASY n-1) and number of days with rain greater than 5 mm (nd > 5) in the 30 days after stabilization of average temperature around 10 °C.

Symptoms disappearance

Concerning the incidence of vines that exhibited foliar symptoms in year n but were symptomless in year n+1 (ASY n+1 subset), which expresses the ability of the plant to hide symptoms, and climatic parameters in the year n+1, no significant relation was found in winter (January–February) and spring (March–April). On the other hand, significant regressions were found in May–June and June–July for all cultivars except Merlot. Best fits were obtained in May–June when ASY n+1 incidences on Sauvignon blanc and Cabernet sauvignon were significantly related with the number of days with maximum temperature above 30°C. The linear model fitted well to these relations: the incidence of plants with hidden symptoms increased as temperature increased (Figure 4). On the other hand, in Cannonau ASY n+1 incidence significant regressions were with minimum and average temperature and showed a good fit to the quadratic model resulting in a narrow parabolic curve (best fit was with minimum temperature, Figure 4): the incidence of plants with hidden symptoms increased as temperature increased starting from 12°C.

Significant relation with rain parameters was found only in the June–July period, when the ASY n+1 incidence on Cabernet sauvignon and Cannonau was related with total rainfall. In the same period, Sauvignon blanc ASY n+1 incidence showed a quadratic relationship with number of rainy days just below the established limits (P = 0.012). The parabolic trend was due to a single data corresponding to 2011. Excluding this point the relationship between the two parameters showed significant regressions. The exponential model was the best in describing the relationships between Sauvignon blanc and Cabernet sauvignon asymptomatic plants and rain, while the relation with Cannonau plants followed a logarithmic model. Regardless of the model, the trend was that more rain in year n+1 led to less vines with hidden symptoms in the same year (Figure 5).

Monthly disease incidences

No relation was found between monthly incidences of new symptomatic plants and climatic parameters in the entire period surveyed. On the contrary, interesting regressions were found considering only incidence of new symptomatic vines in the first and second assessments and in the second and third assessments, for all cultivars except Merlot.

As regard the first and second assessment (between 50% sprouting and the end of June), the best significant regressions were obtained between average minimum temperatures and incidence of new symptomatic vines for all cultivars. In particular, the logarithmic model described better these relations: the higher the temperature the greater the increase in disease incidence (Figure 6).

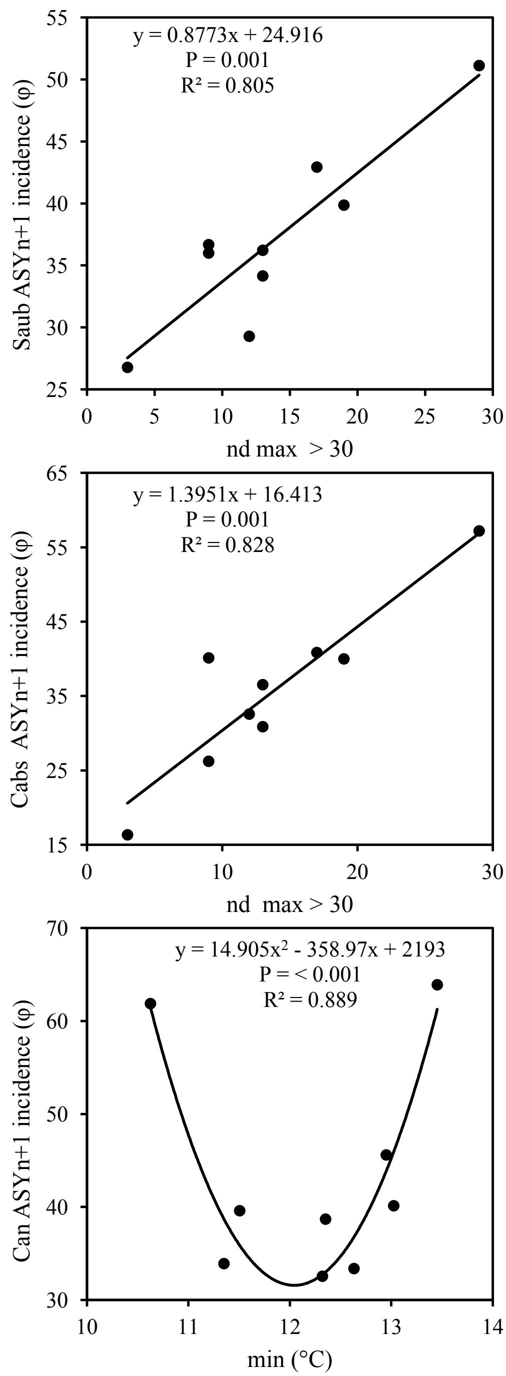

Figure 4. Regressions between arcsin-square root transformed (degrees φ) annual incidence of Sauvignon blanc (Saub), Cabernet sauvignon (Cabs) and Cannonau (Can) plants showing GLSD foliar symptoms in year n but symptomless in year n+1 (ASY n+1) and temperature parameters (nd max > 30 = number of days with maximum temperature above 30 °C; min = average minimum temperature) in the periods May – June of the symptomless year.

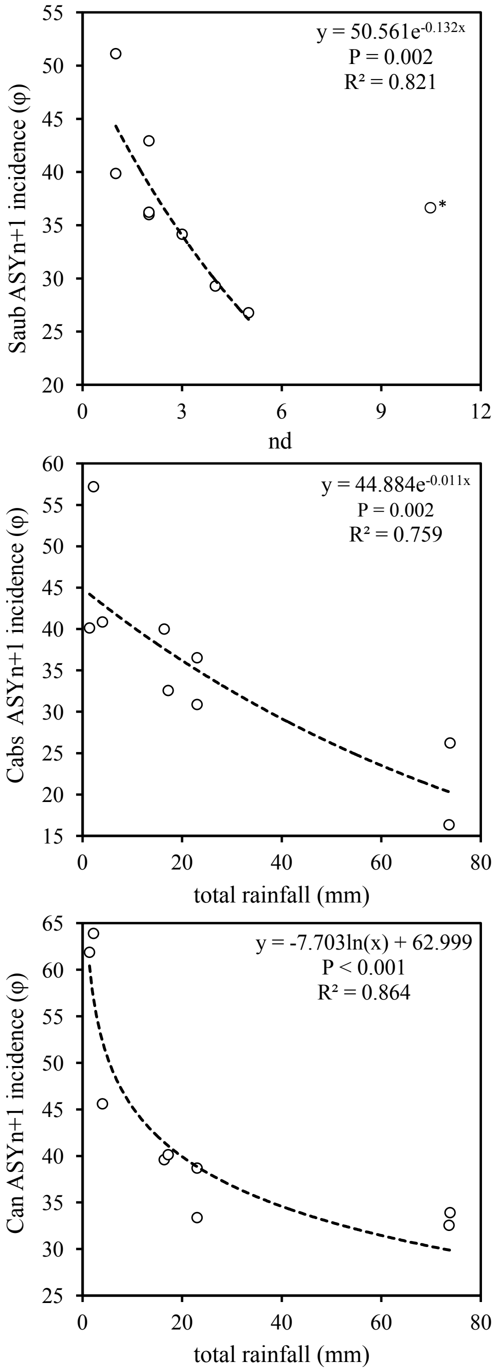

Figure 5. Regressions between arcsin-square root transformed (degrees φ) annual incidence of Sauvignon blanc (Saub), Cabernet sauvignon (Cabs) and Cannonau (Can) plants showing GLSD foliar symptoms in year n but symptomless in year n+1 (ASY n+1) and rainy parameters (nd = number of rainy days; total rainfall) in the period June – July of the symptomless year. Data point marked with asterisk was excluded by the analysis.

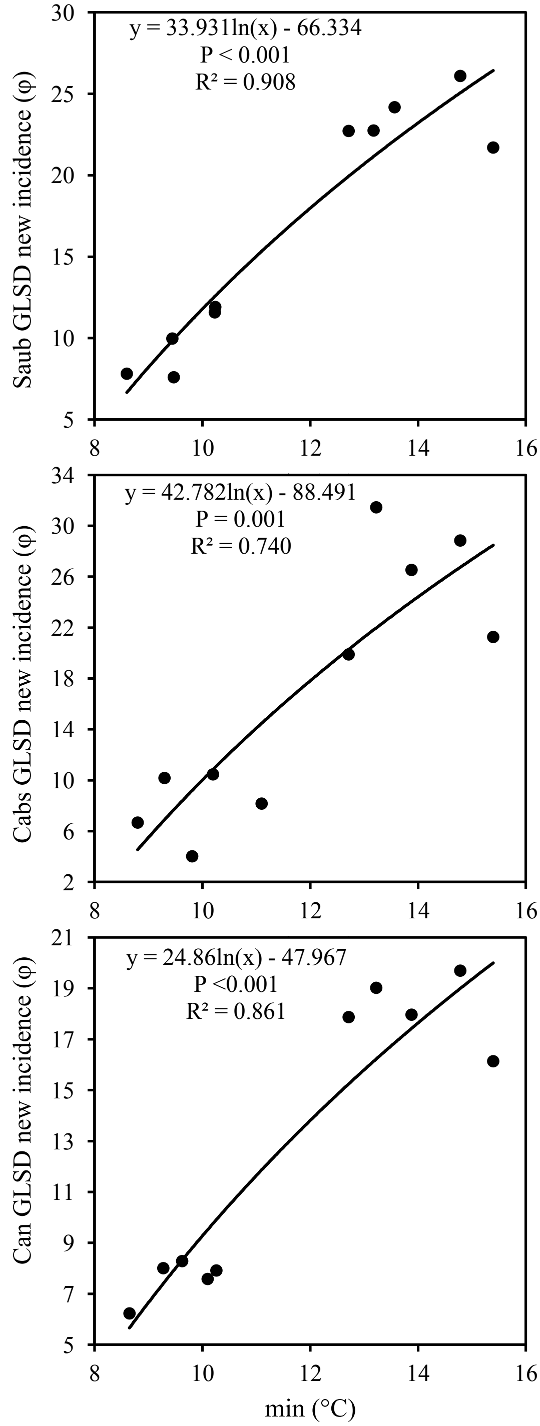

Figure 6. Regressions between arcsin-square root transformed (degrees φ) incidences of new Sauvignon blanc (Saub), Cabernet sauvignon (Cabs) and Cannonau (Can) plants showing GLSD foliar symptoms in the first and second assessment and average minimum temperature from 50% budding to the end of June.

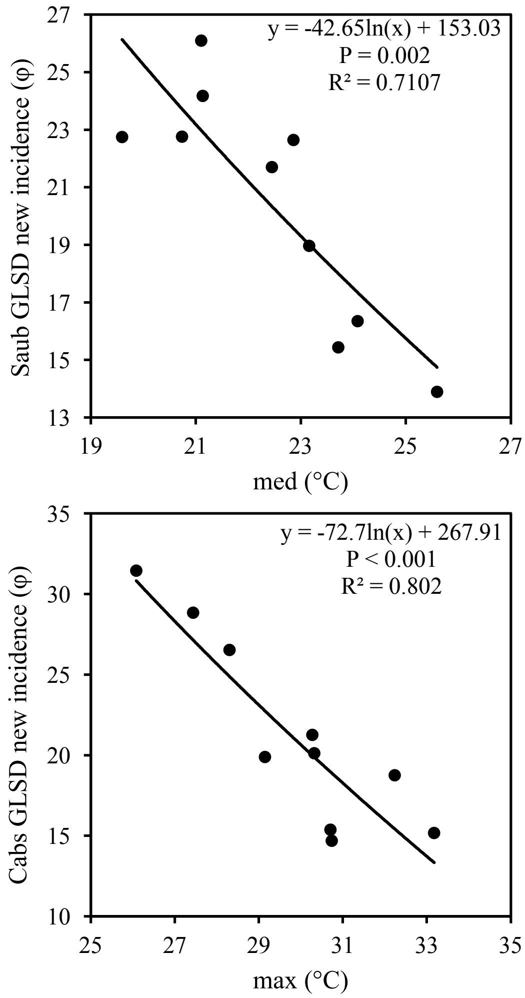

Figure 7. Regressions between arcsin-square root transformed (degrees φ) incidences of new Sauvignon blanc (Saub) and Cabernet sauvignon (Cabs) plants showing foliar symptoms in the second and third assessment and temperature parameters (med = average temperature; max = average maximum temperature) from the end of May to the end of July.

In the second and third assessment (from the end of May to the end of July), incidences of new symptomatic vines were significantly related with medium temperatures for Sauvignon blanc and with maximum temperatures for Cabernet sauvignon. As in the previous period, the logarithmic model fitted to these relationships, but showed the opposite trend: with increasing the temperature the incidence of new symptomatic plants decreased (Figure 7).

Discussion

The incidence of GLSD symptomatic plants, both annual and cumulative, reached very high levels except on Merlot. The low susceptibility of Merlot to esca complex, especially compared to Cabernet sauvignon, has already been reported (Christen *et al.*, 2007; Borgo *et al*. 2008; Quaglia *et al*., 2009; Fussler *et al*., 2008; Bruez *et al*., 2013; Lambert *et al*., 2013; Murolo and Romanazzi 2014; Pouzoulet *et al*., 2014).

As repeatedly reported throughout world's vine-growing areas, and as seen in this study, the annual incidence of foliar symptoms fluctuated through the years. They appeared within the 50% flowering stage (end of May) in all cultivars, with maximum increments recorded in June and partially in July, from fruit setting to veraìson, confirming previous findings (Marchi *et al*., 2006; Lecomte *et al*., 2011; Andreini *et al*., 2014). This is precisely the period in which the plant requires a greater amount of water and nutrients for growth of berries and shoots and can more easily undergo stress conditions by, for example a dysfunction of the xylem apparatus or the circulation of toxins.

Symptom fluctuations between years and monthly increments showed a similar annual trend in the cultivars when symptom assessment was carried out at the same time, making sense of the climate influence. Nevertheless, no relationship was found considering GLSD annual incidence and the climatic parameters along the vegetative season, or considering the four monthly increments and the monthly climatic parameters. In the period after 50% flowering, corresponding to June-August, we found one significant regression with rain and one with temperature, but only for one cultivar. The difficulty in finding a relation between the annual incidence of symptomatic plants and climatic parameters over a fairly long period may depend on the concomitant influence of other factors on symptom expression as observed by other authors (Surico *et al*., 2000). Plant aging alone could justify a change in the onset of symptoms in the 10 years of survey, regardless of the weather conditions.

To find significant and consistent relations valid for most cultivars examined, it was necessary to consider only GLSD incidence fractions and/or short time periods. Good positive regressions were obtained between the incidence of symptomatic plants that were symptomless the previous year (ASY n-1 subset) and rainy parameters in the 30 days after stabilization of average temperatures around 10°C on all cultivars except Merlot. As already mentioned, this temperature was crucial in Sardinia for the start of colonization of pruning wounds by *Pa. chlamydospora* and *Pm. minimum* and infections, which were favored by regularly distributed rainfall (Serra *et al*. 2008). These data would suggest a link between re-infections and symptom expression. Thus, the more the rain conditions favorable to new infections after the pruning, the greater the number of plants that, asymptomatic the previous year, showed symptoms. This evidence emerged also in trials for protection of pruning wounds with *Trichoderma*-based fungicides, where a significant reduction in the number of symptomatic plants was observed, particularly in plants that showed foliar symptoms for the first time, in the treated plots (Reggiori *et al*., 2014).

The ability of the vine to hide symptoms, i.e. incidence of vines symptomatic in year n but asymptomatic in the year n+1 (ASY n+1 subset), was not associated with adverse climatic conditions, particularly low temperature in the coldest period, that may kill or damage the pathogens. Probably, the mild Mediterranean climate is not cold enough to hinder pathogens that remain safe inside their hosts. On the other hand, significant regressions with temperature in the year n+1 were found during the vine growing season. In particular, high temperatures in the period from pre-flowering to veraìson (May–July) were conducive to a greater number of vines with hidden symptoms. As above-mentioned, high temperature causes a partial closure of the stomata resulting in reduced sap flow and probably lower concentrations of toxins in the leaves. In fact, more significant regressions were found with number of days with maximum temperature above 30°C, at least for Sauvignon blanc and Cabernet sauvignon. Significant regressions with rain parameters were found in the driest period (June–July) according to the results of Marchi *et al*., (2006). It would seem that water stress does not favor the onset of symptoms, confirming previous findings (Goutouly, 2007).

The monthly incidence of new symptomatic vines showed significant regressions considering only a short time period and excluding the last increment in August, confirming that events leading to maximum symptom expression were already working by the end of July. Comparing the GLSD incidence increments in the first and second assessments with those in the second and third, it is interesting to observe the opposite relationship with temperature. Starting from the 50% sprouting stage to June the higher the temperature the greater the percentage of new plants that show symptoms in the assessment. On the contrary, from the end of May to the end of July temperature increases correspond to a smaller percentage of newly symptomatic plants. This is consistent with vine water balance and toxin transport by the sap flow.

According to the above, the relationships between climatic factors and disease incidence were affected by cultivars. Variation in incidence of symptom expression might have affected these relationships also with respect to the same climatic factor. For example, only one significant regression, difficult to elucidate, was found for cv. Merlot (see additional chart), that showed low disease incidence. This may be an interesting source of information in future investigations, taking into account its unique behavior compared to the other three cultivars. However, it should not be forgotten that only 7 out of 10 years of assessments were common to all cultivars, three of which had different rootstocks that actively contribute to the plant water balance.

In conclusion, the expression of GLSD symptoms is confirmed to be a complex phenomenon in which climatic conditions are only one of the many factors involved. Apart from the effect of weather on symptom onset in plants asymptomatic the previous year through the influence of annual re-infections, which needs to be clarified, the results of this study indicate that the expression and fluctuation of foliar symptoms seem to be very related to plant phenology and water status. Maximum expression of foliar symptoms occurred in the period between fruit set and veraìson, when plant metabolism directs most of the available resources to shoot and berries growth, leaving few opportunities for the implementation of defense mechanisms. Water deficit, due to low rainfall and high temperatures, appeared to be associated with a reduction in symptom expression. The plant water status can be influenced not only by weather, but also by soil characteristics, genetic traits of cultivars and rootstocks, and vineyard management. Therefore, detailed study of physiological aspects through an interdisciplinary approach would lead to a better understanding of GLSD symptoms expression that might consent to adapt agronomic management to each vineyard in order to limit disease appearance and consequently economic losses.

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