Phytopathologia Mediterranea (2018), 57, 3, 488−505
DOI: 10.14601/Phytopathol_Mediterr-23787

RESEARCH PAPERS - 10TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Rainfall and temperature influence expression of foliar symptoms of grapevine leaf stripe disease (esca complex) in vineyards

Francesco CALZARANO[1], Fabio OSTI[2], Miroslav BARÁNEK[3] and Stefano DI MARCO[2]

[1] Università degli Studi di Teramo, Facoltà di BioScienze e Tecnologie Agro-Alimentari ed Ambientali, Via Renato Balzarini 1, 64100 Teramo, Italy
[2] CNR, IBIMET, Via Gobetti 101, 40129 Bologna, Italy
[3] Mendeleum–Department of Genetics, Faculty of Horticulture, Mendel University in Brno, Valtická 334, Lednice, Czech Republic

Summary. Two vineyards in the Abruzzo Region, central Italy, affected by grapevine leaf stripe disease (GLSD), were monitored for incidence and severity of foliar symptoms of the disease for 21 consecutive years (1994 to 2014). Each year, rainfall and temperature were recorded. Correlations between symptom incidence or severity and rainfall or temperature of different periods of the year or single months were assessed. July rainfall and temperature were highly correlated with incidence and severity of leaf symptoms. The vineyards were characterized by high longevity, vigour and yields, and a particular dynamic of GLSD. In the period following 2005, the year of peak vine mortality, there was a decrease of the expression of foliar symptoms, which was not correlated with both of the climate parameters. Greater mortality occurred for vines showing severe symptoms at the first appearance of GLSD than for vines with low severity.

Key words: rainfall, temperature, mortality, GLSD foliar symptoms.

Introduction

Grapevine trunk diseases are destructive in vineyards. Among these diseases, grapevine leaf stripe disease (GLSD), a syndrome of the esca complex, is the most widespread, occurring in almost all grape growing areas (Fontaine *et al*., 2016). The expression of GLSD in vineyards is characterized by appearance of foliar symptoms and progressive weakening of affected plants. GLSD has been recently described as a tracheomycosis, mainly caused by *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. (*P. minimum* in particular). These fungi colonize the woody tissues of grapevines, causing black streaking and brown necrosis (Mugnai *et al*., 1999; Marchi *et al*., 2001; Calzarano and Di Marco, 2007; Surico, 2009). In most cases, the wood of GLSD diseased vines can affected by white rot, caused by the basidiomycete *Fomitiporia mediterranea*. However, the role of *F. mediterranea* in the formation of foliar symptoms has not been demonstrated, but the basidiomycete is probably involved in vine apoplexy. Therefore, foliar symptoms are probably associated with tracheomycotic fungi (Calzarano and Di Marco, 2007; Surico *et al*., 2008, 2009; Bertsch *et al*., 2013).

The characteristic leaf symptoms of GLSD are chlorosis developing between the leaf veins. Chlorosis can expand and the leaf tissues partially necrotize. Leaves finally assume the tiger-stripe pattern characteristic of the disease. Foliar symptoms are generally associated with wilting of shoots and, in some cases, with black measles of berry skins and withering of the bunches (Mugnai *et al*., 1999; Surico *et al*., 2009).

Plants affected by GLSD may not exhibit symptoms for several years after the first appearance, and produce yields that are similar to that of healthy

Corresponding author: F. Calzarano
E-mail: fcalzarano@unite.it

ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm
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plants (Calzarano *et al.*, 2004). Furthermore, the incidence of foliar symptoms was not correlated with the extent of wood alterations (Calzarano and Di Marco, 2007). In any case, the incidence and severity of GLSD foliar symptoms is associated with quantitative and qualitative yield losses (Calzarano *et al*., 2001, 2004; Bertsch *et al*., 2013). Reduction of symptom expression has been obtained with the use of *Trichoderma* (Di Marco *et al*., 2004), fungicides (Di Marco *et al*., 2011a, 2011b), and foliar fertilizers (Calzarano *et al*., 2014, 2017a; Calzarano and Di Marco, 2018).

Surico *et al.* (2000) investigated the erratic nature of appearance of GLSD symptoms: only a limited number of plants that were symptomatic in a given year exhibited symptoms the following year, and even fewer exhibited symptoms in each of the years of their study. Therefore, the proportion of symptomatic plants assessed in a given year was not a reliable indicator of the incidence of the disease in affected vineyards.

Further studies confirmed that several consecutive years of symptom assessment are necessary to define the cumulative incidence (diseased plants showing symptoms at least in a given year of investigation) as a more accurate determination of GLSD incidence in each vineyard (Di Marco *et al*., 2011a; Zanzotto *et al.*, 2013). Plants may not express symptoms for 8-10 consecutive years after the first symptom appearance.

Vineyards surveyed in Tuscany (central Italy) showed that most of the vines (>70%) were already symptomatic for GLSD at the end of July each year. The same was observed in Sicilian and Sardinian vineyards (Surico *et al*., 2000). In contrast, in other regions of central Italy, such as Abruzzo, symptoms on most plants appeared later, in August and September (Calzarano *et al.*, 2016). However, the dynamics of foliar symptom expression during the grapevine growing season indicated that the mechanisms involved in the symptom development are effective until the end of July each year (Surico *et al*., 2000).

The erratic nature of GLSD symptoms has been related to environmental and/or cultural factors (Mugnai *et al*., 1999; Surico *et al*., 2000; Marchi *et al*., 2006; Calzarano *et al*., 2009; Surico *et al*., 2010; Gramaje *et al*., 2018; Lecomte *et al.*, 2018). Surico *et al*. (2000) demonstrated that cool and rainy summers were favourable to foliar symptom expression. These results were confirmed by Marchi *et al*. (2006). In that study, rainfall throughout the first part of the growing season seemed to increase symptom expression. A statistically significant correlation between rainfall in July and May-July, and expression of leaf symptoms was observed in two of three vineyards, for a period of 4 and 8 years of assessment (Marchi *et al.* 2006).

These results are consistent with observations in Tuscany vineyards, where greatest foliar symptom expression was noticed in vineyard areas subject to water accumulation and/or waterlogging (Surico *et al*, 2000). It was then hypothesized that increased soil water availability may increase vine transpiration, with increased translocation of phytotoxic substances to the leaves (Marchi *et al*., 2006). Although the mechanisms leading to formation of GLSD leaf symptoms are still debated, the most agreed hypothesis is based on production of phytotoxic substances by tracheomycotic fungi, or on substances produced during wood degradation induced by the pathogens (Sparapano *et al*., 1998; Mugnai *et al*., 1999; Evidente *et al*., 2000; Tabacchi *et al*., 2000). These substances can be translocated to grapevine canopy, causing alterations of leaf photosynthesis and plant defense responses (Petit *et al.*, 2006; Andolfi *et al*., 2011; Bertsch *et al*., 2013; Calzarano *et al*., 2016, 2017a, 2017b).

The increased expression of leaf symptoms in rainy seasons was confirmed in a further 4-year study, where a large amount of water, regularly supplied to 20-year old esca-affected vines transplanted in pots, favoured the expression of foliar symptoms. These appeared in most plants every year for the first three years of the trial (Surico *et al*., 2010). In the fourth year, symptoms in plants regularly irrigated appeared before, and to a greater extent, than observed in plants irrigated only at the beginning of water stress. The appearance of symptoms in potted plants also anticipated what occurred in the vineyard, probably because of the constant irrigation carried out in April and May (Surico *et al*., 2010). In the same experiment, in addition to the role of water, relatively high temperatures assessed in June (when the mechanisms leading to the foliar symptom expression can occur) seemed to favour the expression of symptoms (Surico *et al*., 2010). However, in the absence of consistent data, the role of temperature in the expression of foliar symptoms remains to be verified.

The aim of the present study was to confirm and extend knowledge of the influence of rainfall on GLSD symptom expression, and to assess the role of temperature in the expression of leaf symptoms on grapevines affected by this disease.

Materials and methods

Evaluation of leaf symptoms

The leaf symptoms of GLSD were recorded for 21 consecutive years in two vineyards of cv. Trebbiano d'Abruzzo on rootstock 420A. Both vineyards are now 40 years old, and are located in Controguerra and Giulianova, two parts of the province of Teramo (central Italy). The Controguerra vineyard was trained to the Geneva Double Courtain (GDC) system, with a planting pattern of 2m within rows and 4 m between rows, and had average grape yield of 13 to 17 kg per vine. The Giulianova vineyard was trained to the Tendone system, with a vine spacing of 3 × 3 m, and an average annual yield of 20 kg per vine. Each year, approx. 500 vines in the Controguerra and 2,000 in the Giulianova vineyard were individually inspected for symptom. The symptom assessments were carried out on all the vines, though vines tested in trials aimed at GLSD control were excluded from analyses to avoid interference of these vines on the foliar symptom expression.

Each year, leaf symptoms were detected in the second half of September, at the time of maximum seasonal symptom incidence and severity in the investigated area. In a given year, the number of plants showing foliar symptoms were identified and compared to the total number of plants in the vineyard. This value was the percentage of symptomatic vines in a given year, and represented the annual incidence of GLSD. For each year of observation, the severity of the disease was assessed on each plant using an arbitrary scale of 0 to 5, where 0 = no leaf symptoms; 1 = 1–10%; 2 = 11–30%; 3 = 31–50%; 4 = 51–70%; or 5 = 71–100% of leaves with symptoms. Severity for each year was calculated according to the following formula: SN × 100 / (Y × Z), where SN = sum of all the values of the arbitrary severity scale attributed to each symptomatic vine of the vineyard in that year; Y = number of plants observed; and Z = maximum value of the symptom scale (McKinney, 1923).

Rainfall and temperature measurements

Daily rainfall and temperature data for the 21 years of leaf symptom assessments were recorded from weather stations by: i) "Servizio Idrografico e Mareografico" - Abruzzo Region; ii) "Centro Agrometeorologico" (CAR) - Abruzzo Region; and iii) "Direzione L. L. P. P. e Protezione Civile" - Abruzzo Region. Data were recorded hourly by the weather station equipment. All data were grouped on a daily base. The weather stations were located approx. 2 km from the vineyards as the crow flies.

Correlations between leaf symptoms and rainfall or temperature

In both vineyards, for each year, sums of daily rainfall and active temperature (Amerine and Winkler, 1944; Winkler, 1962) were calculated. Data of both climatic parameters were grouped for different periods or considered for a single month, corresponding to grapevine growth stages, as follows: i) April-May, "Bud burst" (BBCH 09) to "Inflorescence clearly visible" (BBCH 53); ii) April-June, "Bud burst" (BBCH 09) to "Fruit set" (BBCH 71); iii) April-July, "Bud burst" (BBCH 09) to "Berries beginning to touch" (BBCH 77); ìv) April-August, "Bud burst" (BBCH 09) to "Berries developing colour" (BBCH 83); v) May-June, "Inflorescence clearly visible" (BBCH 53) to "Fruit set" (BBCH 71); vi) May-July, "Inflorescence clearly visible" (BBCH 53) to Berries beginning to touch" (BBCH 77); vii) May-August, "Inflorescence clearly visible" (BBCH 53) to "Berries developing colour" (BBCH 83); viii) June-July, "Fruit set" (BBCH 71) to "Berries beginning to touch" (BBCH 77); ix) June-August, "Fruit set" (BBCH 71) to "Berries developing colour" (BBCH 83); x) July-August, "Berries beginning to touch" (BBCH 77) to "Berries developing colour" (BBCH 83); and the months of April, May, June, July and August.

The periods and single months were during the grapevine growing season, except for in September because symptoms did not develop further in the investigated vineyards. The periods and single months were chosen according to symptom expression previously assessed at various growth stages in the same vineyards (Calzarano *et al*., 2016). In particular, the first foliar symptoms appeared sporadically in July. From the end of July (pre-bunch closure), the number of symptomatic vines increased rapidly, particularly between veraison and harvest, reaching the maximum in the second half of September, just before the harvest. It was therefore likely that the environmental factors associate with symptom expression applied in the April to August period, as also shown by Marchi *et al.* (2006).

The sum of active temperatures (SAT) was calculated using the Winkler Index (Amerine and Winkler, 1944; Winkler, 1962). This was calculated for the period April 1 to August 31, using the formula: IW = $\Sigma_{01.04/31.08}$ (Tmed-10). For each day, the Index indicates the thermal time useful for grapevine growth, as the difference between the average daily temperature and 10°C, the minimum grapevine growth temperature. For each period, the daily thermal units are then summed. Only temperatures above zero were considered. For each vineyard, temperature data were correlated with the incidence and severity of the GLSD symptoms.

For each year, the annual data of symptom incidence and severity were compared with sums of rainfall or sums of active temperature for each period. The climatic data for each of these periods were correlated with the incidence and severity of leaf symptoms, according to the Pearson correlation coefficient (from -1 to 1) and its probability was also calculated and reported. Pearson correlation coefficient was considered significant at $P = 0.05$. Statistical analyses were carried out using SAS version 9.3 (SAS Institute Inc.).

Symptom severity and vine mortality

In both vineyards, for each year of assessment, the percentage of vines in each symptom class was calculated to evaluate the dynamics of severity of each class of foliar symptom throughout the 21-year period of this study. Annual and cumulative vine mortality was also assessed in both vineyards (Di Marco and Osti, 2009). Mortality of the symptomatic vines was also assessed in relation to the level of symptom severity shown during the first appearance of symptoms.

Results

Evaluation of leaf symptoms

In the Controguerra vineyard, the greatest annual incidences of GLSD were recorded in the period from 1994 to 2006, with an annual incidence in most cases greater than 20% and greatest at 35.6% in 1999 (Table 1). Also in the 1994–2006 period, the severity of foliar symptoms followed a similar trend of annual incidence, and was generally never less than 12%, with greatest severity of 22.9% in 1999 (Table 1). During the following period of 2007–2014, annual incidence and particularly severity generally decreased (Table 1). The lowest annual incidence and severity were recorded in 2007, at 7.3 and 4.0%, respectively (Table 1). In the 2007–2014 period, symptom severity was from 4.0 to 8.6%, except for 2010 (10.5%) and 2011 (11.8%), and were generally less than recorded in the period 1994–2006.

In the Giulianova vineyard, differences in symptom expression assessed in 1994–2006 compared to in 2007–2014 were more obvious than in Controguerra vineyard (Table 3). In the period 1994–2006, annual incidence was from 5.8% in 2004 to 25.9% in 1999, and symptom severity was from 4.2% in 2004 to 16.8% in 1999 (Table 3). In the period 2007–2014, annual incidence was from 2.8% in 2010 to 6.4% in 2009, and symptom severity was from 1.7% in 2012 to 3.8% in 2008 (Table 3). The greatest annual incidence and symptom severity both occurred in 1999, whereas the least annual incidence was recorded in 2012, and least severity was recorded in 2008 (Table 3).

Rainfall and temperature measurements

In both vineyards, rainfall showed high variation from year to year over the 21 years of observations (Table 1 and 3). The greatest average rainfall for the growth season periods (April–August) was recorded in both vineyards during the 1994–2006 interval, in 1999, 2002 and 2004, from 309.2 to 431.2 mm for the Controguerra vineyard, and 238.4 mm to 392.6 mm in the Giulianova vineyard (Tables 1 and 3). In the 2007–2014 interval, the years with greatest average rainfall were, in decreasing order, 2013, 2009 and 2012 in Controguerra (from 377.6 to 509.6 mm), and 2013, 2014 and 2010, in Giulianova (from 282.0 to 436.0 mm) (Tables 1 and 3). In both vineyards, the greatest rainfall for the 21 years of observations (1994–2014) was in 2013, when 509.6 mm was recorded in Controguerra and 436.0 mm in Giulianova (Tables 1 and 3). In both vineyards, low rainfall was recorded more frequently in the 1994–2006 interval, with least amounts in 1994 in the Controguerra vineyard (121.8 mm) and in 2003 in the Giulianova vineyard (92.8 mm) (Tables 1 and 3).

In both vineyards, the average monthly rainfall for the April-August periods, for the 1994–2006 interval, were greatest in April (Controguerra 58.1 mm and Giulianova 58.6 mm). The least average monthly rainfall were in July in Controguerra (40.7 mm) and in June in Giulianova (34.2 mm). In the 2007–2014 interval the average monthly rainfall was normally greater than recorded in the 1994–2006 interval, with the exception of August, the month with the lowest rainfall,

Table 1. Controguerra vineyard: Total rainfall for different periods in 21 years, and annual incidence and severity of GLSD foliar symptoms.

Year	Rainfall (mm)															Foliar symptoms	
	Apr-May	Apr-June	Apr-July	Apr-Aug	May-Jun	May-July	May-Aug	Jun-July	Jun-Aug	July-Aug	April	May	June	July	August	Incidence (%)	Severity (%)
1994	58.6	112.8	112.8	121.8	70.2	70.2	79.2	54.2	63.2	9.0	42.6	16.0	54.2	0.0	9.0	11.5	6.6
1995	101.4	145.0	169.6	230.0	80.6	105.2	165.6	68.2	128.6	85.0	64.4	37.0	43.6	24.6	60.4	24.5	13.4
1996	101.8	137.0	179.4	286.4	110.4	152.8	259.8	77.6	184.6	149.4	26.6	75.2	35.2	42.4	107.0	26.0	13.3
1997	109.2	157.0	221.8	261.2	72.6	137.4	176.8	112.6	152.0	104.2	84.4	24.8	47.8	64.8	39.4	23.4	12.9
1998	69.8	87.4	118.4	162.4	54.0	85.0	129.0	48.6	92.6	75.0	33.4	36.4	17.6	31,0	44.0	28.2	13.5
1999	114.2	194.6	368.8	431.2	118.6	292.8	355.2	254.6	317.0	236.6	76.0	38.2	80.4	174.2	62.4	35.6	22.9
2000	68.6	78.4	116.4	122.2	24.6	62.6	68.4	47.8	53.6	43.8	53.8	14.8	9.8	38.0	5.8	24.4	11.5
2001	122.0	155.2	166.6	166.6	74.6	86.0	86.0	44.6	44.6	11.4	80.6	41.4	33.2	11.4	0.0	24.5	14.6
2002	162.4	197.4	256.4	309.2	138.6	197.6	250.4	94.0	146.8	111.8	58.8	103.6	35.0	59,0	52.8	29.3	17.5
2003	44.4	106.8	121.2	141.0	68.4	82.8	102.6	76.8	96.6	34.2	38.4	6.0	62.4	14.4	19.8	15.9	7.2
2004	165.2	249.2	288.6	326.8	149.8	189.2	227.4	123.4	161.6	77.6	99.4	65.8	84.0	39.4	38.2	25.4	14.4
2005	101.0	134.0	153.2	237.2	69.2	88.4	172.4	52.2	136.2	103.2	64.8	36.2	33.0	19.2	84.0	27.2	13.2
2006	43.2	107.6	118.8	149.6	75.4	86.6	117.4	75.6	106.4	42.0	32.2	11.0	64.4	11.2	30.8	15.0	7.5
2007	101.8	133.8	245.4	287.6	97.2	208.8	251.0	143.6	185.8	153.8	36.6	65.2	32.0	111.6	42.2	7.3	4.0
2008	101.0	202.4	225.4	225.4	142.8	165.8	165.8	124.4	124.4	23.0	59.6	41.4	101.4	23.0	0.0	9.6	4.0
2009	126.0	304.2	367.0	433.2	191.6	254.4	320.6	241.0	307.2	129.0	112.6	13.4	178.2	62.8	66.2	17.3	7.0
2010	73.2	127.6	145.2	208.6	91.6	109.2	172.6	72.0	135.4	81.0	36.0	37.2	54.4	17.6	63.4	26.1	10.5
2011	48.2	96.4	160.6	163.4	79.8	144.0	146.8	112.4	115.2	67.0	16.6	31.6	48.2	64.2	2.8	22.4	11.8
2012	229.4	245.8	357.8	377.6	121.0	233.0	252.8	128.4	148.2	131.8	124.8	104.6	16.4	112.0	19.8	18.1	6.3
2013	257.6	343.4	354.0	509.6	309.2	319.8	475.4	96.4	252.0	166.2	34.2	223.4	85.8	10.6	155.6	16.4	6.4
2014	214.0	268.4	314.4	324.4	170.8	216.8	226.8	100.4	110.4	56.0	97.6	116.4	54.4	46.0	10.0	20.9	8.6

Table 2. Rainfall vs GLSD annual symptom incidence or severity correlations at the Controguerra vineyard: Pearson coefficients and values of Pearson coefficient probability for different periods for 1994–2014, 1994–2006 and 2007–2014.

Foliar symptoms	Year interval	Parameter	Apr-May	Apr-June	Apr-July	Apr-Aug	May-Jun	May-July	May-Aug	Jun-July	Jun-Aug	July-Aug	April	May	June	July	August
Incidence	1994–2014	Pearson coefficient	0.03	-0.10	0.03	0.11	-0.17	-0.01	0.08	0.02	0.14	0.33	0.12	-0.04	-0.24	0.24	0.22
		Probability	0.899	0.673	0.922	0.643	0.447	0.980	0.722	0.927	0.552	0.148	0.601	0.873	0.296	0.299	0.336
	1994–2006	Pearson coefficient	**0.63**	0.46	**0.69**	**0.75**	0.38	**0.68**	**0.72**	0.52	**0.64**	**0.77**	0.40	**0.57**	-0.12	**0.72**	0.51
		Probability	***0.015***	0.113	***0.012***	***0.021***	0.208	***0.011***	***0.015***	0.064	***0.015***	***0.032***	0.177	***0.037***	0.689	***0.016***	0.074
	2007–2014	Pearson coefficient	-0.04	-0.09	-0.23	-0.16	-0.12	-0.32	-0.18	-0.34	-0.22	-0.21	0.01	-0.06	-0.10	-0.31	0.03
		Probability	0.910	0.807	0.560	0.671	0.760	0.390	0.630	0.352	0.562	0.577	0.983	0.885	0.799	0.397	0.933
Severity	1994–2014	Pearson coefficient	-0.05	-0.15	0.03	0.08	-0.22	0.01	0.06	0.11	0.16	0.33	0.08	-0.11	-0.21	0.33	0.14
		Probability	0.828	0.526	0.915	0.742	0.348	0.989	0.798	0.639	0.493	0.138	0.727	0.633	0.363	0.148	0.549
	1994–2006	Pearson coefficient	**0.71**	**0.62**	**0.85**	**0.84**	0.53	**0.82**	**0.80**	**0.69**	**0.72**	**0.79**	0.51	**0.58**	0.09	**0.82**	0.41
		Probability	***0.003***	***0.006***	***0.018***	***0.016***	0.059	***0.008***	***0.002***	***0.001***	***0.001***	***0.002***	0.070	***0.024***	0.747	***0.007***	0.176
	2007–2014	Pearson coefficient	-0.30	-0.34	-0.41	-0.38	-0.27	-0.45	-0.33	-0.33	-0.30	-0.29	-0.25	-0.19	-0.14	-0.25	-0.10
		Probability	0.421	0.354	0.249	0.291	0.469	0.186	0.375	0.370	0.415	0.444	0.508	0.607	0.713	0.514	0.801

Pearson coefficients significant at $P = 0.05$ are in bold. Significant values of probability are in bold- italics.

Table 3. Giulianova vineyard: Total rainfall for different periods in 21 years, and annual incidence and severity of GLSD foliar symptoms.

Year	Rainfall (mm)															Foliar symptoms	
	Apr-May	Apr-June	Apr-July	Apr-Aug	May-Jun	May-July	May-Aug	Jun-July	Jun-Aug	July-Aug	April	May	June	July	August	Incidence (%)	Severity (%)
1994	63.8	109.0	116.4	125.4	68.0	75.4	84.4	52.6	61.6	16.4	41.0	22.8	45.2	7.4	9.0	7.7	5.1
1995	86.2	96.4	137.8	203.8	50.8	92.2	158.2	51.6	117.6	107.4	45.6	40.6	10.2	41.4	66.0	7.5	5.6
1996	69.4	79.4	105.0	168.2	49.8	75.4	138.6	35.6	98.8	88.8	29.6	39.8	10.0	25.6	63.2	16.5	7.9
1997	120.4	138.8	160.6	224.4	52.6	74.4	138.2	40.2	104.0	85.6	86.2	34.2	18.4	21.8	63.8	18.6	10.1
1998	133.8	141.4	157.6	215.2	101.2	117.4	175.0	23.8	81.4	73.8	40.2	93.6	7.6	16.2	57.6	12.3	5.5
1999	128.4	199.2	367.6	392.6	93.4	261.8	286.8	239.2	264.2	193.4	105.8	22.6	70.8	168.4	25.0	25.9	16.8
2000	41.2	69.2	94.2	103.2	44.0	69.0	78.0	53.0	62.0	34.0	25.2	16.0	28.0	25.0	9.0	12.2	6.7
2001	129.4	146.4	150.4	152.6	51.4	55.4	57.6	21.0	23.2	6.2	95.0	34.4	17.0	4.0	2.2	8.3	4.4
2002	172.0	188.8	261.2	383.0	103.4	175.8	297.6	89.2	211.0	194.2	85.4	86.6	16.8	72.4	121.8	14.6	9.8
2003	27.8	53.8	82.4	92.8	27.2	55.8	66.2	54.6	65.0	39.0	26.6	1.2	26.0	28.6	10.4	7.4	4.3
2004	119.2	178.4	197.6	238.4	102.8	122.0	162.8	78.4	119.2	60.0	75.6	43.6	59.2	19.2	40.8	5.8	4.2
2005	94.2	137.6	150.8	234.6	73.2	86.4	170.2	56.6	140.4	97.0	64.4	29.8	43.4	13.2	83.8	13.9	8.6
2006	50.0	119.0	120.0	165.6	77.2	78.2	123.8	70.0	115.6	46.6	41.8	8.2	69.0	1.0	45.6	9.7	5.5
2007	46.6	67.6	71.2	81.0	43.0	46.6	56.4	24.6	34.4	13.4	24.6	22.0	21.0	3.6	9.8	3.5	1.7
2008	110.0	129.6	148.2	148.2	63.6	82.2	82.2	38.2	38.2	18.6	66.0	44.0	19.6	18.6	0.0	5.5	3.8
2009	123.4	159.6	173.6	208.8	53.6	67.6	102.8	50.2	85.4	49.2	106.0	17.4	36.2	14.0	35.2	6.4	3.6
2010	117.8	130.4	222.2	282.0	100.2	192.0	251.8	104.4	164.2	151.6	30.2	87.6	12.6	91.8	59.8	2.8	1.9
2011	73.0	111.2	187.0	187.4	70.6	146.4	146.8	114.0	114.4	76.2	40.6	32.4	38.2	75.8	0.4	4.4	3.4
2012	134.4	140.8	183.8	188.6	48.4	91.4	96.2	49.4	54.2	47.8	92.4	42.0	6.4	43.0	4.8	3.1	1.7
2013	111.2	198.0	342.4	436.0	182.8	327.2	420.8	231.2	324.8	238.0	15.2	96.0	86.8	144.4	93.6	3.9	2.5
2014	250.6	303.0	361.2	370.0	223.2	281.4	290.2	110.6	119.4	67.0	79.8	170.8	52.4	58.2	8.8	4.3	2.7

Table 4. Rainfall vs GLSD annual symptom incidence or severity correlations at the Giulianova vineyard: Pearson coefficients and values of Pearson coefficient probability for different periods for 1994–2014, 1994–2006 and 2007–2014.

Foliar symptoms	Year interval	Parameter	Apr-May	Apr-June	Apr-July	Apr-Aug	May-Jun	May-July	May-Aug	Jun-July	Jun-Aug	July-Aug	April	May	June	July	August
Incidence	1994–2014	Pearson coefficient	0.003	0.03	0.13	0.22	-0.16	0.03	0.14	0.18	0.29	0.32	0.30	-0.22	0.06	0.21	0.31
		Probability	0.990	0.907	0.456	0.259	0.495	0.746	0.436	0.282	0.125	0.078	0.183	0.333	0.798	0.360	0.165
	1994–2006	Pearson coefficient	0.35	0.39	**0.65**	**0.63**	0.21	**0.65**	**0.62**	**0.63**	**0.68**	**0.69**	0.45	0.09	0.13	**0.73**	0.25
		Probability	0.241	0.183	***0.017***	***0.020***	0.490	***0.016***	***0.025***	***0.020***	***0.011***	***0.008***	0.125	0.766	0.682	***0.005***	0.409
	2007–2014	Pearson coefficient	0.06	0.13	-0.10	-0.14	-0.13	-0.27	-0.27	-0.21	-0.21	-0.33	0.52	-0.27	0.19	-0.40	-0.18
		Probability	0.865	0.739	0.959	0.925	0.73	0.488	0.441	0.546	0.519	0.225	0.094	0.476	0.610	0.266	0.630
Severity	1994–2014	Pearson coefficient	0.04	0.10	0.24	0.31	-0.11	0.12	0.22	0.31	0.39	0.40	0.36	-0.22	0.15	0.32	0.32
		Probability	0.857	0.663	0.224	0.120	0.642	0.467	0.259	0.104	0.099	0.092	0.106	0.342	0.508	0.151	0.161
	1994–2006	Pearson coefficient	0.40	0.51	**0.79**	**0.76**	0.29	**0.79**	**0.73**	**0.80**	**0.83**	**0.80**	**0.56**	0.05	0.27	**0.86**	0.27
		Probability	0.178	0.074	***0.001***	***0.003***	0.331	***0.001***	***0.005***	***0.001***	***0.0005***	***0.0009***	***0.045***	0.870	0.370	***0.0002***	0.372
	2007–2014	Pearson coefficient	0.05	0.13	0.02	-0.04	-0.02	-0.08	-0.12	-0.01	-0.08	-0.18	0.32	-0.15	0.24	-0.14	-0.20
		Probability	0.894	0.731	0.767	0.922	0.954	0.996	0.893	0.758	0.970	0.858	0.394	0.695	0.532	0.705	0.602

Pearson coefficients significant at $P = 0.05$ are in bold. Significant values of probability are in bold- italics.

especially in the Giulianova vineyard, with 26.6 mm (Tables 1 and 3).

The greatest temperatures as sums of active temperature for the April-August periods, over the 21 years of observations, were recorded in 2003, with 1918.9°C in Controguerra and 1913.5°C in Giulianova (Tables 5 and 7). In both vineyards, the years with the greatest average sums of active temperatures were recorded in 1994–2006 and 2007–2014 intervals. The years with the lowest temperatures, i.e. 1995, 1996, 1997, 1999, 2001, 2004 and 2005, were all in the 1994–2006 interval, except for 2014 in Controguerra vineyard, with 1475.3°C (Tables 5 and 7).

For each month of the April-August period, the average monthly sums of active temperatures were similar in both vineyards in the two intervals, 1994–2006 and 2007–2014. The exception was the month of April, when greater temperatures were recorded in the 2007–2014 interval (126.0°C in Controguerra and 131.7°C in Giulianova), compared to 92.3°C in Controguerra and 92.1°C in Giulianova for the 1994–2006 interval (Tables 5 and 7).

Correlations between leaf symptoms and rainfall or temperature

The correlations were weak or absent between rainfall and leaf symptoms for each of the different periods of each year from 1994 to 2014 (Tables 1, 2, 3 and 4). A decrease in the annual incidence and severity of GLSD symptoms occurred since 2007, when the vines were 29 years old. Therefore, analyses were carried out considering two main intervals, 1994–2006 and 2007–2014.

In the Controguerra vineyard, in 1994–2006, for each of the annual periods and single months, except for May–June, April, June and August, rainfall was significantly correlated with both symptom incidence and severity. In the period 1994–2006, Pearson coefficients ranged from 0.57 to 0.85 in the periods or months significantly correlated with rainfall. In April–June and in June–July the rainfall and symptom correlation was significant only for the symptom severity (Tables 1 and 2).

In the Giulianova vineyard, in the 1994–2006 interval, the rainfall-incidence and the rainfall-severity correlations were statistically significant for the same periods as for the Controguerra vineyard, except for April–May and April–June. The significant Pearson coefficients values ranged from 0.62 to 0.86. Among single months only July rainfall was significantly correlated with both symptom incidence and severity, with Pearson coefficients of 0.73 for incidence and 0.86 for severity (Tables 3 and 4). April rainfall was significantly correlated with symptom severity (Pearson coefficient = 0.56 : Tables 3 and 4).

In both vineyards the strongest rainfall-symptom correlations were in July each year, and in the periods including this month.

Also in both vineyards, in the 2007–2014 period, no statistically significant correlations were recorded between rainfall and symptoms (Tables 1, 2, 3 and 4).

In the Controguerra vineyard, in the 1994–2006 period, in the periods April–July, April–August, May–August, June–July, June–August, and July–August, significant inverse correlations were recorded between the sums of active temperatures and symptom incidence or severity; the Pearson coefficient values ranged from -0.54 to -0.61 (Tables 5 and 6). In particular, in April–July and June–July the correlations between temperature and symptom incidence were not significant. Similarly, the correlations for May–August temperature-symptoms severity were also not significant. Among single months, only the July sum of temperatures was significantly correlated with both symptom incidence and severity, with Pearson coefficient values of -0.61 (Tables 5 and 6).

In the Giulianova vineyard, in the 1994–2006 period, the correlation between sum of active temperatures and symptoms was verified only for July, with correlation coefficients of -0.61 for symptom incidence and of -0.49 for symptom severity (Tables 7 and 8).

In the Controguerra vineyard, also in 1994–2014, an inverse and statistically significant correlation was detected between sum of active temperatures and both incidence and severity of symptoms, with Pearson coefficients ranging from -0.47 to -0.51, detected for the periods April–July, April–August and July–August (Tables 5 and 6). In May–August and June–August only the correlations between temperature and symptom incidence were statistically significant, with values of -0.43 for May–August and -0.44 for June–August (Tables 5 and 6). In the 1994–2014 period, only the sum of temperatures for July was significantly correlated with either symptom incidence or severity, with Pearson coefficients of -0.51 for incidence and -0.53 for severity (Tables 5 and 6).

In the Giulianova vineyard, in the 1994–2014 period, only temperature-symptom correlations for July–August and July were statistically significant, with

Table 5. Controguerra vineyard: Sums of active temperatures for different periods, and annual incidence and severity of GLSD foliar symptoms.

Year	Sums of active temperatures (°C) (Winkler Index) and GLSD foliar symptoms															Foliar symptoms	
	Apr-May	Apr-June	Apr-July	Apr-Aug	May-Jun	May-July	May-Aug	Jun-July	Jun-Aug	July-Aug	April	May	June	July	August	Incidence (%)	Severity (%)
1994	359.3	721.8	1217.0	1802.4	616.1	1111.3	1696.6	857.7	1443.0	1080.5	105.8	253.6	362.5	495.2	585.4	11.5	6.6
1995	305.9	619.8	1103.2	1503.9	534.2	1017.6	1418.3	797.3	1198.0	884.2	85.6	220.3	313.9	483.5	400.7	24.5	13.4
1996	355.8	728.0	1151.5	1560.0	618.8	1042.3	1450.8	795.7	1204.2	832.0	109.2	246.7	372.2	423.5	408.5	26.0	13.3
1997	266.8	620.6	1019.7	1426.1	586.7	985.8	1392.2	752.9	1159.3	805.5	33.9	232.9	353.8	399.1	406.4	23.4	12.9
1998	317.6	685.2	1161.6	1639.2	583.6	1060.0	1537.6	844.0	1321.6	954.0	101.6	216.0	367.6	476.4	477.6	28.2	13.5
1999	347.3	702.1	1111.1	1555.3	612.4	1021.4	1465.5	763.8	1208.0	853.2	89.7	257.6	354.8	409.0	444.2	35.6	22.9
2000	435.8	818.2	1230.1	1726.5	684.2	1096.0	1592.4	794.3	1290.7	908.3	134.1	301.8	382.4	411.9	496.4	24.4	11.5
2001	328.8	666.6	1118.9	1651.0	600.9	1053.2	1585.3	790.1	1322.2	984.4	65.7	263.1	337.8	452.3	532.1	24.5	14.6
2002	342.1	741.3	1164.1	1573.0	645.1	1067.9	1476.8	822.1	1230.9	831.7	96.2	245.9	399.2	422.9	408.9	29.3	17.5
2003	399.7	870.7	1359.1	1918.9	783.3	1271.7	1831.5	959.4	1519.2	1048.2	87.4	312.3	471.0	488.5	559.8	15.9	7.2
2004	251.5	592.2	1047.0	1497.9	514.1	968.9	1419.8	795.5	1246.4	905.8	78.1	173.4	340.7	454.8	451.0	25.4	14.4
2005	357.8	723.2	1182.5	1561.5	640.4	1099.7	1478.7	824.8	1203.7	838.3	82.8	275.0	365.5	459.3	379.0	27.2	13.2
2006	382.1	723.0	1193.3	1590.5	593.0	1063.4	1460.6	811.3	1208.5	867.6	130.0	252.1	340.9	470.4	397.2	15.0	7.5
2007	448.2	829.3	1343.4	1788.6	668.0	1182.1	1627.3	895.3	1340.4	959.3	161.3	286.9	381.1	514.2	445.2	7.3	4.0
2008	358.8	708.2	1160.7	1632.7	588.4	1040.9	1512.8	801.9	1273.9	924.5	119.9	239.0	349.4	452.5	472.0	9.6	4.0
2009	428.0	765.9	1222.9	1695.3	657.1	1114.1	1586.5	794.9	1267.3	929.4	108.8	319.2	337.9	457.0	472.4	17.3	7.0
2010	341.3	692.4	1180.6	1615.9	586.2	1074.5	1509.8	839.4	1274.7	923.6	106.2	235.1	351.1	488.3	435.3	26.1	10.5
2011	424.4	797.2	1233.3	1734.8	635.3	1071.4	1572.9	809.0	1310.5	937.6	161.9	262.5	372.9	436.1	501.5	22.4	11.8
2012	327.6	747.7	1257.4	1758.7	641.7	1151.4	1652.8	929.8	1431.1	1011.1	106.0	221.7	420.1	509.7	501.4	18.1	6.3
2013	337.4	658.3	1116.5	1581.5	528.7	986.9	1451.9	779.1	1244.1	923.3	129.6	207.8	320.9	458.2	465.1	16.4	6.4
2014	317.6	667.2	1061.9	1475.3	553.0	947.7	1361.1	744.3	1157.7	808.2	114.2	203.4	349.6	394.7	413.5	20.9	8.6

Table 6. Sums of active temperatures vs symptom incidence or severity correlations at the Controguerra vineyard: Pearson coefficients and values of Pearson coefficient probability for different periods for 1994–2014, 1994–2006 and 2007–2014.

Foliar symptoms	Year interval	Parameter	Apr-May	Apr-June	Apr-July	Apr-Aug	May-Jun	May-July	May-Aug	Jun-July	Jun-Aug	July-Aug	April	May	June	July	August
Incidence	1994–2014	Pearson coefficient	-0.40	-0.34	**-0.49**	**-0.51**	-0.18	-0.39	**-0.43**	-0.40	**-0.44**	**-0.50**	-0.40	-0.20	-0.10	**-0.51**	-0.36
		Probability	0.067	0.131	***0.025***	***0.024***	0.432	0.077	***0.045***	0.071	***0.042***	***0.024***	0.065	0.378	0.667	***0.024***	0.112
	1994–2006	Pearson coefficient	-0.28	-0.28	-0.49	**-0.55**	-0.24	-0.48	**-0.54**	-0.53	**-0.57**	**-0.61**	-0.21	-0.23	-0.20	**-0.61**	-0.48
		Probability	0.365	0.365	0.086	***0.046***	0.435	0.096	***0.049***	0.058	***0.037***	***0.021***	0.497	0.442	0.521	***0.021***	0.096
	2007–2014	Pearson coefficient	-0.38	-0.35	-0.39	-0.35	-0.27	-0.33	-0.29	-0.24	-0.21	-0.25	-0.35	-0.28	-0.07	-0.31	-0.06
		Probability	0.381	0.349	0.290	0.346	0.473	0.382	0.435	0.531	0.543	0.500	0.347	0.451	0.891	0.404	0.884
Severity	1994–2014	Pearson coefficient	-0.38	-0.34	**-0.49**	**-0.48**	-0.17	-0.38	-0.40	-0.42	-0.42	**-0.47**	-0.38	-0.15	-0.12	**-0.53**	-0.30
		Probability	0.089	0.137	***0.030***	***0.031***	0.473	0.084	0.069	0.054	0.055	***0.034***	0.087	0.505	0.591	***0.032***	0.189
	1994–2006	Pearson coefficient	-0.34	-0.35	**-0.56**	**-0.56**	-0.30	-0.54	-0.53	**-0.58**	**-0.54**	**-0.54**	-0.28	-0.28	-0.26	**-0.61**	-0.40
		Probability	0.261	0.246	***0.038***	***0.041***	0.329	0.053	0.056	***0.024***	***0.050***	***0.049***	0.359	0.369	0.388	***0.011***	0.191
	2007–2014	Pearson coefficient	-0.08	-0.10	-0.25	-0.20	-0.13	-0.28	-0.22	-0.28	-0.21	-0.27	0.01	-0.12	-0.06	-0.39	0.01
		Probability	0.823	0.788	0.512	0.589	0.730	0.460	0.551	0.456	0.570	0.480	0.973	0.756	0.869	0.285	0.984

Pearson coefficients significant at $P = 0.05$ are in bold. Significant values of probability are in bold- italics.

Table 7. Giulianova vineyard: Sums of active temperatures for different periods, and annual incidence and severity of GLSD foliar symptoms.

Year	Sums of active temperatures (°C) (Winkler Index) and GLSD foliar symptoms															Foliar symptoms	
	Apr-May	Apr-June	Apr-July	Apr-Aug	May-Jun	May-July	May-Aug	Jun-July	Jun-Aug	July-Aug	April	May	June	July	August	Incidence (%)	Severity (%)
1994	333.9	669.6	1136.2	1651.2	580.7	1047.3	1562.4	802.3	1317.4	981.7	88.9	245.0	335.7	466.6	515.1	7.7	5.1
1995	288.1	586.9	1061.3	1463.3	513.7	988.2	1390.1	773.2	1175.2	876.4	73.2	215.0	298.8	474.5	402.0	7.5	5.6
1996	332.3	699.8	1095.4	1502.3	605.4	1001.0	1407.9	763.1	1170.1	802.5	94.4	237.9	367.6	395.6	407.0	16.5	7.9
1997	284.8	647.7	1049.8	1465.8	608.8	1010.9	1426.9	765.0	1181.0	818.1	38.9	245.9	362.9	402.1	416.0	18.6	10.1
1998	359.9	737.9	1187.9	1700.3	613.5	1063.5	1575.9	828.0	1340.4	962.4	124.4	235.5	378.0	450.0	512.4	12.3	5.5
1999	395.3	752.3	1177.6	1661.0	634.6	1059.9	1543.4	782.3	1265.7	908.8	117.7	277.7	357.0	425.3	483.5	25.9	16.8
2000	417.0	770.0	1156.0	1607.0	639.7	1025.7	1476.7	739.0	1190.0	837.0	130.4	286.7	353.0	386.0	451.0	12.2	6.7
2001	286.0	584.2	1055.7	1523.1	528.7	1000.2	1467.6	769.7	1237.1	938.9	55.5	230.5	298.2	471.5	467.4	8.3	4.4
2002	343.4	737.1	1158.8	1561.2	641.3	1063.0	1465.4	815.4	1217.8	824.1	95.8	247.6	393.7	421.7	402.4	14.6	9.8
2003	414.0	878.8	1363.8	1913.5	781.1	1266.1	1815.8	949.8	1499.5	1034.7	97.8	316.3	464.8	485	549.7	7.4	4.3
2004	245.5	578.2	1017.0	1450.8	505.1	943.8	1377.6	771.5	1205.3	872.6	73.2	172.3	332.8	438.8	433.8	5.8	4.2
2005	349.9	711.8	1160.2	1546.7	623.8	1072.2	1458.8	810.3	1196.8	835.0	88.0	262.0	361.9	448.4	386.6	13.9	8.6
2006	365.0	704.6	1166.7	1576.3	585.5	1047.7	1457.3	801.8	1211.4	871.8	119.1	245.9	339.6	462.2	409.6	9.7	5.5
2007	442.3	825.2	1316.9	1766.2	677.3	1169.1	1618.3	874.6	1323.9	941.0	147.9	294.5	382.9	491.8	449.3	3.5	1.7
2008	364.8	701.9	1167.3	1631.5	571.9	1037.3	1501.6	802.5	1266.7	929.7	130.0	234.9	337.1	465.4	464.3	5.5	3.8
2009	434.3	777.7	1240.8	1715.9	654.9	1118.1	1593.2	806.6	1281.7	938.3	122.8	311.5	343.4	463.2	475.1	6.4	3.6
2010	349.8	696.5	1175.1	1608.5	581.7	1060.3	1493.7	825.4	1258.7	912.0	114.8	235.0	346.7	478.7	433.4	2.8	1.9
2011	410.7	781.2	1268.8	1744.6	624.8	1112.4	1588.2	858.1	1334.0	963.5	156.4	254.3	370.5	487.6	475.9	4.4	3.4
2012	352.5	664.8	1168.4	1665.5	545.5	1049.0	1546.1	815.9	1313.0	1000.7	119.4	233.1	312.4	503.6	497.1	3.1	1.7
2013	441.7	776.0	1235.5	1703.6	645.3	1104.8	1572.9	793.8	1261.9	927.6	130.7	311.0	334.3	459.5	468.2	3.9	2.5
2014	348.4	707.7	1121.1	1549.0	576.4	989.8	1417.7	772.7	1200.6	841.3	131.3	217.1	359.3	413.4	427.9	4.3	2.7

Table 8. Sums of active temperatures vs symptom incidence or severity correlations at the Giulianova vineyard: Pearson coefficients and values of Pearson coefficient probability for different periods for 1994–2014, 1994–2006 and 2007–2014.

Foliar symptoms	Year interval	Parameter	Apr-May	Apr-June	Apr-July	Apr-Aug	May-Jun	May-July	May-Aug	Jun-July	Jun-Aug	July-Aug	April	May	June	July	August
Incidence	1994-2014	Pearson coefficient	-0.15	-0.02	-0.26	-0.27	0.15	-0.18	-0.21	-0.32	-0.30	**-0.47**	-0.35	0.07	0.19	**-0.65**	-0.20
		Probability	0.525	0.952	0.251	0.236	0.502	0.446	0.367	0.154	0.188	***0.031***	0.113	0.745	0.407	***0.002***	0.393
	1994–2006	Pearson coefficient	0.30	0.27	0.03	-0.04	0.26	-0.02	-0.08	-0.25	-0.23	-0.39	0.16	0.33	0.16	**-0.61**	-0.16
		Probability	0.322	0.383	0.920	0.898	0.398	0.965	0.782	0.420	0.454	0.190	0.591	0.281	0.599	***0.021***	0.601
	2007–2014	Pearson coefficient	0.30	0.24	0.06	0.12	0.25	0.04	0.11	-0.28	-0.10	-0.10	0.08	0.31	0.005	-0.35	0.22
		Probability	0.418	0.531	0.884	0.756	0.499	0.911	0.767	0.451	0.788	0.793	0.825	0.404	0.994	0.346	0.565
Severity	1994–2014	Pearson coefficient	-0.15	-0.04	-0.26	-0.27	0.12	-0.17	-0.21	-0.30	-0.30	**-0.44**	-0.34	0.05	0.15	**-0.59**	-0.21
		Probability	0.516	0.883	0.265	0.235	0.602	0.451	0.355	0.182	0.191	***0.043***	0.135	0.823	0.506	***0.010***	0.357
	1994–2006	Pearson coefficient	0.27	0.22	0.03	-0.05	0.21	-0.01	-0.09	-0.22	-0.23	-0.35	0.14	0.30	0.10	**-0.49**	-0.18
		Probability	0.384	0.476	0.904	0.841	0.492	0.948	0.747	0.473	0.459	0.246	0.643	0.335	0.719	***0.048***	0.547
	2007–2014	Pearson coefficient	0.13	0.11	-0.05	0.01	0.07	-0.11	-0.04	-0.26	-0.12	-0.13	0.21	0.07	0.03	-0.35	0.16
		Probability	0.734	0.764	0.894	0.988	0.854	0.768	0.923	0.484	0.760	0.738	0.57	0.861	0.93	0.346	0.664

Pearson coefficients significant at $P = 0.05$ are in bold. Significant values of probability are in bold- italics.

Pearson coefficients -0.47 for July–August and -0.65 for July for symptom incidence, and -0.44 and -0.59 for the same periods for symptom severity (Tables 7 and 8).

In the 2007–2014 period, in both vineyards, no consistent statistically significant correlations were recorded between the sums of active temperatures and incidence or severity of symptoms (Tables 5, 6, 7, and 8).

Level of symptom severity and vine mortality

Decreases of symptom expression were observed in the Giulianova and Controguerra vineyards during the years of this study. The number of vines of each severity rating class decreased from 1994 to 2014 (Figure 1). This decrease was most evident for the vines in greater classes of symptom severity (Figure 1).

In both vineyards, the percentage of annual vine mortality decreased after 2006, with the maximum mortality occurring in 2005, in Controguerra, and in 2003 and in 2005 in Giulianova. Cumulative vine mortality stabilized after 2007 (Figure 2).

In both vineyards, in the 1994-2014 period, the percentages of dead vines which showed GLSD symptoms in a previous year, increased with increasing the levels of severity of symptoms observed at first appearance (Figure 3).

Discussion

This study was carried out in Trebbiano d'Abruzzo vineyards trained to the expanded trellis system e.g. "Tendone", with each vine trunk being 2 m high and with four permanent branches from which abundant foliage developed. The vineyards are characterized by extended longevity, vigour and yields. These characteristics may have to particular GLSD dynamics, with plants remaining asymptomatic for several years after the first appearance of symptoms. These characteristics, as well as cultural practices, can play important roles in the expression of GLSD (Lecomte *et al*., 2011; Pancher *et al*., 2012; Travadon *et al*., 2016; Kraus *et al*., 2017).

Rainfall plays a significant role in the modulation of leaf symptom expression (Surico *et al*., 2000; Marchi *et al*., 2006; Surico *et al*., 2010; Latinovic and Latinovic, 2017; Serra *et al*., 2018). In the present study, results obtained in the period 1994–2006 confirmed the influence of rainfall in the first half of each summer, particularly in July, on symptoms incidence and severity, as has already been ascertained by Marchi *et al*. (2006). In the Controguerra and Giulianova vineyards the rainfall-symptoms correlations were greatest in July and in the periods including this month, confirming the importance of July rainfall conditions for expression of foliar symptoms.

In contrast to the hypothesis of Surico *et al*. (2010), in both vineyards in the period of 1994 to 2006, inverse correlations between sums of active temperatures and symptom expression were recorded for July each year. In the Controguerra vineyard, these correlations also involved periods including July, while in the Giulianova vineyard significant correlations were detected only for July. The inverse July correlations between sums of active temperatures and symptoms were verified, and in some periods including July, also for the 21 years of observations, from 1994 to 2014. This strengthens the hypothesis that temperatures in (Northern Hemisphere) July play an important role in the expression of GLSD symptoms.

July rainfall was a key factor in the symptom expression. It is important to note that in the vineyards observed in this study the first symptoms of GLSD appeared regularly at the end of July.

Growing seasons with high rainfall in July characterized by low temperatures, favoured higher expression of foliar symptoms. Fresh and rainy summers are favorable to GLSD, probably increasing plant physiological processes, maybe including the movement of fungal toxins to the leaves (Di Marco and Osti, 2009; Surico *et al*., 2000, 2009, 2010).

The symptoms-rainfall and symptoms-temperature correlations assessed in 1994–2006, were no longer found in the following period, 2007–2014. Although rainfall and temperature appeared to be favourable, this period has been characterized by a decrease of symptom expression.

The lack of a correlation between the leaf symptoms and wood deterioration was demonstrated by Calzarano and Di Marco (2007). On the other hand, foliar symptom expression of esca was associated with the amount of internal necrosis in French vineyards (Maher *et al.* 2012). This should not be surprising because of the difference in the vineyard characteristics and the peculiarities of disease, with several factors involved. It is common belief that a plant with severe symptoms will be easier to die respect to a plant with low or no foliar symptoms. The present study demonstrated this belief: plants with high severity symp-

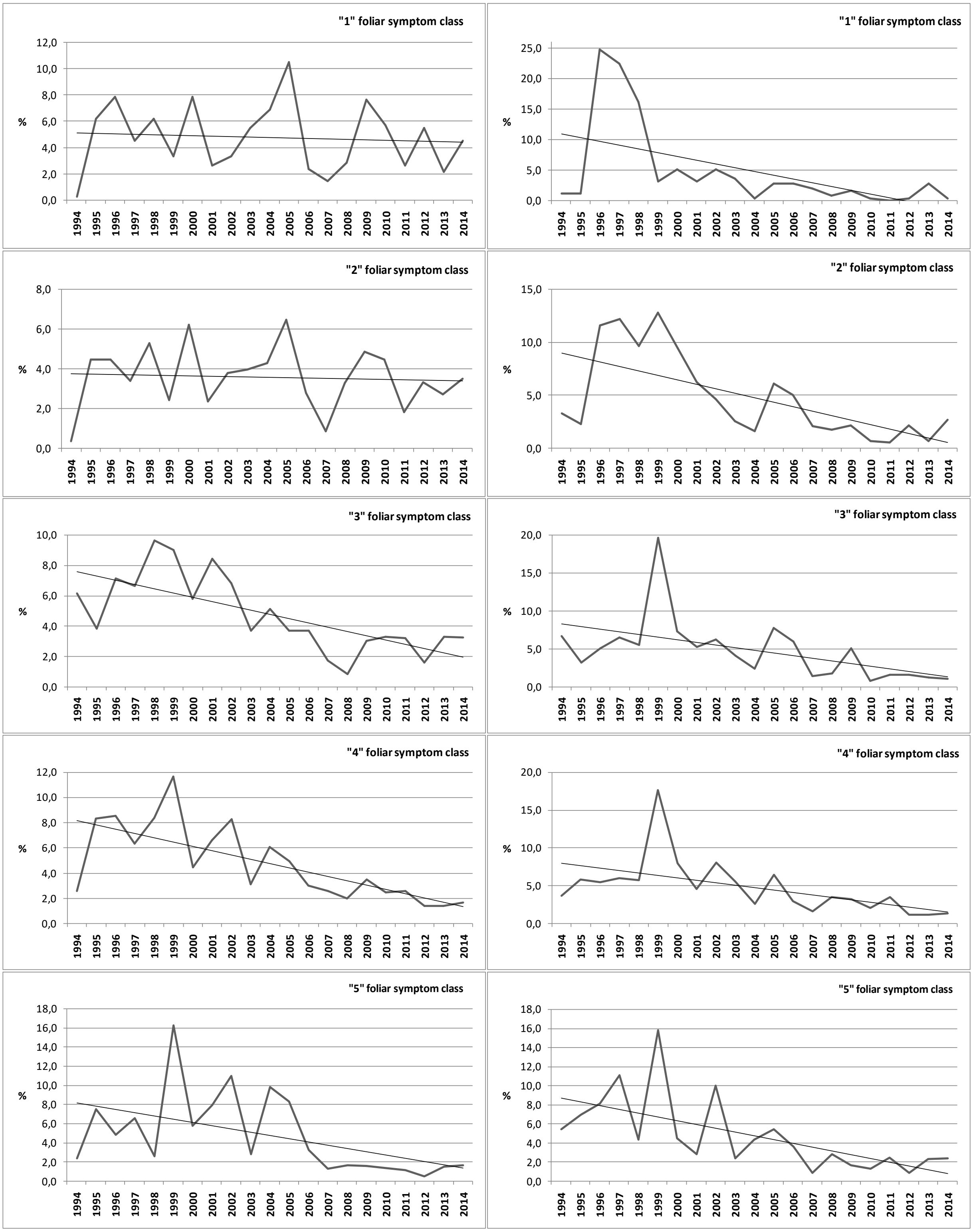

Figure 1. Percentages of grapevines in different GLSD foliar symptom classes from 1994 to 2014 at the Controguerra vineyard (left) and Giulianova vineyard (right).

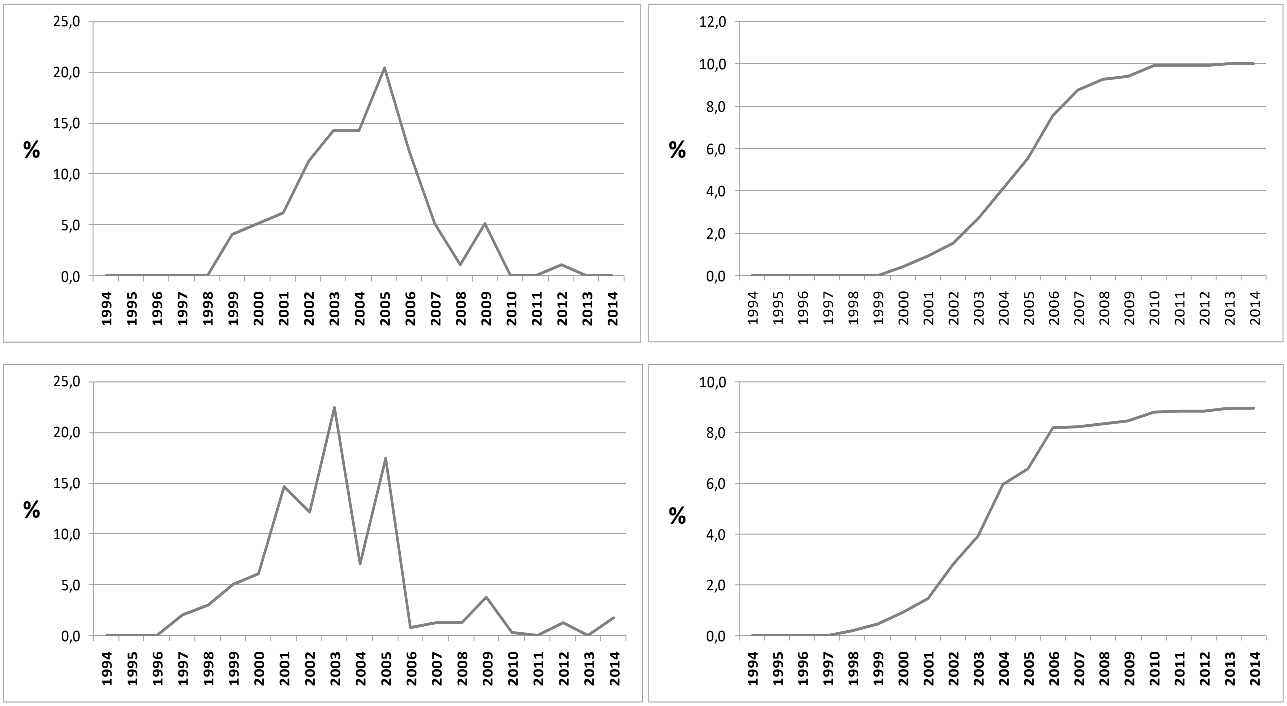

Figure 2. Annual and cumulated vine mortality at Controguerra vineyard (above) and Giulianova vineyard (below).

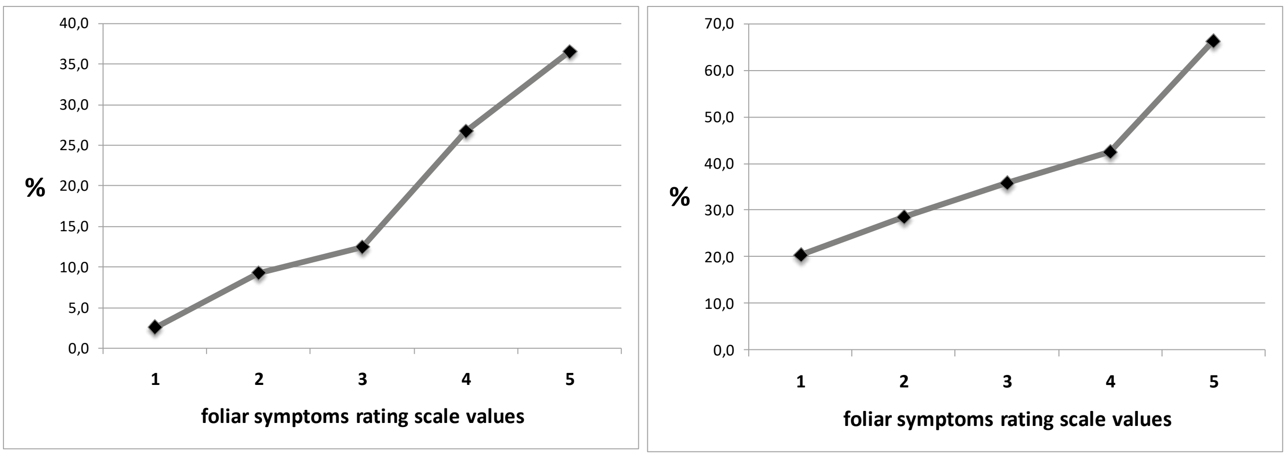

Figure 3. Mortality of GLSD symptomatic vines, in relation to the level of symptom severity shown during the first appearance of leaf symptoms, at the Controguerra vineyard (left) and Giulianova vineyard (right).

toms at the first appearance of the disease, died more frequently than plants showing low severity symptoms. Therefore, foliar symptom is particularly important in the outcome of GLSD and attempts aimed at decreasing in foliar symptom expression not only reduced the yield loss but also might contribute to reduce the possibility that the plant die (Calzarano *et al*., 2004, 2014, 2017a; Di Marco and Osti, 2009; Di Marco *et al*., 2011a, 2011b; Calzarano and Di Marco, 2018). Furthermore, in this study the highest mortality of the vines was recorded in the years just before 2006. In the following years the vineyards were characterized by low mortality, low severity of expression, and vine symptoms no longer appeared related to rainfall or temperature. It might not to be excluded that mature vines, although still in full production, reduced their attitude to respond to the disease by external symptoms.

This study showed once again the complex interaction among the expression of the disease, and agronomical and environmental factors (Mugnai *et al*., 1999; Bertsch *et al*., 2013). Finally our results confirmed the correlation between rainfall and symptom expression and demonstrated the role of temperature on annual incidence and severity of foliar symptoms, highlighting age-related differences in the level of this correlation, over a many-year observation period.

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Accepted for publication: December 10, 2018