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Logistic modelling of summer expression of esca symptoms on tolerant and susceptible cultivars in Bordeaux vineyards

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ABSTRACT

The seasonal dynamics of esca leaf symptom development were monitored and modelled over 10 years (from 2004 to 2006, 2012 to 2014, and 2018 to 2021) in eleven vineyards near Bordeaux (France) and on five cultivars, including three susceptible and two tolerant cultivars. Field observations performed once or twice a week from the end of May to mid-September confirmed i) the evolution over time of esca leaf symptoms, ii) the presence under the bark of a discolored xylem longitudinal stripe with nonfunctional vessels, and iii) a gradual increase in the number of symptomatic plants within each vineyard. Of the three models tested, nonlinear logistic regression was the best fitting curve, showing a clear and systematic progressive sigmoidal pattern of cumulative esca leaf symptom observations regardless of 'vineyard*year' situation. Relationships with climatic data confirmed that all periods of symptom expression corresponded to the warmest and driest period of each vegetative season. Examinations of key dates corresponding to four threshold levels of cumulative incidence of leaf symptomatic vines [S1 (first observed symptoms), S10 %, S50 % and S90 %] showed that tolerant cultivars (Merlot noir and Malbec) generally developed leaf symptoms later than susceptible cultivars (Cabernet-Sauvignon, Cabernet franc, and Sauvignon blanc). A variance analysis and a principal component analysis (PCA) confirmed that compared to susceptible cultivars, tolerant cultivars were associated with increased temperature sums above 10 °C from 1st January, reaching the same symptom thresholds S1 and S10 % and with more cumulative rainfall at the S1 stage. Overall, this study reveals the key role of temperature as a triggering factor for esca symptom expression in relation to fungal activity. The results indicate that the S10 % stage can be used as a discriminant variable to separate cultivars according to their susceptibility. Finally, logistic modelling can be used as a descriptive and analytical tool to study the seasonal dynamics of esca.

KEYWORDS: epidemiology, symptomatology, leaf symptom dynamics, logistic model, climate

INTRODUCTION

With *Eutypa* and *Botryosphaeria* diebacks, esca disease is currently one of the main causes of decline in mature vines in many grape-growing regions worldwide (Bertsch *et al.*, 2013; Kaplan *et al.*, 2016; Gramaje *et al.*, 2018), particularly in some European countries (Bruez *et al.*, 2013; Mondello *et al.*, 2018; Guérin-Dubrana *et al.*, 2019). Since the end of the last millennium, this fungal disease has affected the perennial parts of vine stocks. Esca re-emergence can be associated with different factors, such as poor plant material quality, climate change and/or certain cultural practices (Gramaje and Armengol, 2011; Travadon *et al.*, 2016; Lecomte *et al.*, 2011; Lecomte *et al.*, 2018; Fischer and Peighami-Ashnaei, 2019).

Esca is characterised by the development of three distinct types of symptoms (Arnaud and Arnaud, 1931; Lecomte *et al.,* 2012). The first type comprises wood lesions, cankers and/or necrosis within the grapevine wood structure. Necrotic lesions are very diverse in size, shape and amount of discoloration (Larignon and Dubos, 1997; Mugnai *et al.*, 1999; Maher *et al.,* 2012). Necrosis generally originates from wounds, particularly from grafting points or pruning wounds. It results from the colonisation of several wood-inhabiting fungi, such as *Phaoemoniella chlamydospora* and *Phaeoacremonium minimum*, often considered pioneering agents (Larignon and Dubos, 1997; Mugnai *et al.*, 1999), and *Fomitiporia mediterranea,* the main basidiomycete fungus responsible for white rot, wood degradation often considered the last stage of wood decay (Mugnai *et al.*, 1999; Cortesi *et al.*, 2000; Maher *et al.*, 2012; Moretti *et al.*, 2021). However, basidiomycetes can also act as primary pathogens (Sparapano *et al.*, 2000; Brown *et al.*, 2020). In addition, grapevine wood can be characterised by the presence of a large and complex community of microorganisms, including many other fungi and bacteria putatively involved in the onset of esca (Bruez *et al.*, 2015; Bruez *et al.*, 2016; Bruez *et al.*, 2020; Del Frari *et al.*, 2019; Del Frari *et al.*, 2021; Moretti *et al.*, 2021). The second type of symptom associated with esca is known as 'xylem stripe' or 'brown stripe', and it was no longer included for a long time in many descriptions and was reintroduced only recently by Lecomte *et al*. (2012). This symptom corresponds to a superficial and longitudinal vascular disorder located just under the bark of the trunk and/or the arm(s). It develops at foliar symptom onset and is only located on the symptomatic shoots and the associated vascular pathway. This stripe has also been described as a symptom caused by *Botryosphaeria* species (Larignon *et al.,* 2001). However, these pathogens are not always isolated from such lesions (Lecomte *et al*., 2014a), and the mechanisms behind the formation of this unusual symptom, such as foliar symptoms, are still not fully understood. The third kind of symptom comprises leaf symptoms that appear mostly in early summer (Lecomte *et al.*, 2012), allowing growers and technicians to externally identify the disease. These symptoms are also very variable and differ between black or white cultivars in terms of speed of symptom development and severity (Lecomte *et al*., 2014b). Leaf symptoms are characterised by gradual discoloration, marginal and/or interveinal scorching/drying zones, chlorosis, wilting or sudden collapse and leaf fall, this last very often characterising the most severe form, also called the 'apoplectic form'. According to several authors (Lecomte *et al*., 2008; Maher *et al*., 2012; Ouadi *et al*., 2019), their appearance on mature vines in summer coincides with and results from the development of inner necrosis, which reaches a critical threshold volume. Finally, it has been recently shown that symptomatic esca leaves and shoots are impacted by high levels of hydraulic failure (i.e., loss of hydraulic conductivity) associated with xylem vessel occlusions (gels and/or tyloses) (Bortolami *et al*., 2019; Bortolami *et al.*, 2021a; Bortolami *et al.*, 2023). However, leaf symptom onset and related triggering factors explaining their sudden summer occurrence are still a matter for study and scientific debate (Bortolami *et al*., 2019; Bortolami *et al*., 2021a; Pouzoulet *et al*., 2019; Claverie *et al*., 2020; Del Frari *et al*., 2021).

Leaf symptom onset seems to be part of a more general pattern of regular and progressive temporal development of the disease, regardless of the vineyard. In every ten of the 'vineyard*year' combination cases studied by Lecomte *et al.* (2012), the cumulative incidence of symptomatic vines showed, notably, a clear sigmoidal pattern throughout the summer period. Based on this finding a mathematical model was sought to illustrate the intra-annual progressive appearance of esca-symptomatic vines. Logistic regression models are often used in pathological studies to obtain good descriptions of polycyclic diseases by including multiple disease cycles within a growing season (Madden *et al*., 2017; Nutter, 1997). Therefore, different logistic functions have been used in plant epidemiology for different purposes, in particular for risk assessment and decision-making in disease management (Hughes, 2017), as well as in disease prevalence or symptom incidence studies (e.g., Mila *et al*., 2004; Hay *et al*., 2022). However, to date, no model has yet been proposed to characterise and best fit the temporal evolution of the incidence of esca in vineyards, either for typical epidemics in which symptoms are foliar or for other grapevine trunk diseases. Thus, current knowledge of esca in grapevine clearly shows that there is still a strong need to clarify its etiology, and particularly to better define the underlying mechanisms that drive the summer onset of leaf and vascular symptoms.

Many abiotic factors can influence pathogen and/or disease development, but in particular the role of climatic variables and environmental changes has recently been reviewed (Fischer and Peighami-Ashnaei, 2019; Songy *et al*., 2019). Links between climate effects and esca symptomatology are not always clear; Surico *et al.* (2000), for example, did not detect any specific conditions conducive to esca expression depending on rainfall and/or air temperature parameters, and Andreini *et al.* (2014) did not find any direct relationship between the occurrence of esca symptoms and environmental factors. Information also varies greatly depending on the epidemiological variables of interest: emergence of symptoms, annual incidence, variability of expression depending on the year and severity.

In field observations, temperature and especially rainfall are the most studied climatic factors. In the literature, the most well-known effect of climate is related to the severest form of foliar symptoms, the ‘apoplectic form’. This form has been correlated with the occurrence of heavy rain (e.g., during a storm) followed by a dry and warm period (Galet, 1995; Dubos, 2002) or hot wind, and thus possibly an excess of water in the soil, likely leading to “an imbalance between transpiration and absorption” (Viala, 1893; Surico *et al*., 2006). However, this association between apoplexy and high temperature after heavy rain was not reported in an earlier epidemiological study (Surico *et al.*, 2000), which indicated that early apoplexy mainly occurred under water stress and high temperatures. Such temporal observations of apoplexy are probably not mutually exclusive. Surico *et al*. (2000) also reported that a cool and rainy summer seemed more favourable for the development of the chronic form of esca, while a hot and dry summer seemed more favourable for the severe form. These trends are in line with observations made by Rives (1926) in France, who reported that dry summers were not favourable for esca, and with those of Braccini *et al.* (2005) and Marchi *et al*. (2006) in Italy, where rainfall seemed positively related to the incidence of esca foliar symptoms. Similarly, Larignon (2009) found that years with high esca incidence were associated with a rainy spring, while years with lower incidence were characterised by a dry spring. Similarly, a survey of six Bordeaux vineyards showed a strong positive correlation within each vineyard between the sum of rain over the period May-August and esca expression (Guérin-Dubrana *et al*., 2012). Latinovic and Latinovic (2017) also confirmed an increase in esca incidence in Montenegro in 2016 following an unusual amount of rainfall in spring (in addition to the vine age effect). The influence of rainfall, notably, in the first half of each summer (i.e., in July) was confirmed by other authors in a 21-year survey (1994–2014) in two vineyards located in central Italy (Calzarano *et al*., 2018); they found an inverse correlation between temperature and symptom expression for the month of July. All these reports indicate that the amount of rainfall before and in early summer is a key factor for esca expression. This finding is supported by the results of recent experimental work under controlled conditions, which showed that plant water status was a key driver of esca leaf symptom development, suggesting that water variability impacts esca pathogenesis (Bortolami *et al*., 2021b). However, rainfall is a factor that cannot be separated from water availability, which depends on soil, slope or irrigation (Surico *et al*., 2000; Destrac-Irvine *et al.,* 2007; Robotic and Bosancic, 2007; Calvo-Garrido *et al*., 2021). The role of temperature is more difficult to understand; it has been described as a triggering factor for symptom expression (Lecomte *et al.,* 2012), as a favouring factor (Ouadi *et al.*, 2019; Calvo-Garrido *et al*., 2021) and as decreasing leaf symptom incidence (Calzarano *et al*., 2018; Serra *et al.,* 2018; Calvo-Garrido *et al*., 2021).

In this context, our major objectives were (i) to document the different stages of the evolution of esca foliar symptoms during the season, as well as the presence of longitudinal stripes under the bark of esca-diseased vines - a symptom often neglected (Lecomte *et al*., 2012), ii) to model the progressive onset of esca foliar symptoms over the summer, and iii) to examine the relationships between the seasonal development of temperature as a triggering factor of leaf symptom development and cultivar susceptibility. This study was conducted in different vineyards near Bordeaux in three survey periods: 2004–2006 (data from Lecomte *et al.*, 2012), 2012–2014, and 2018–2021.

MATERIALS AND METHODS

1. Experimental vineyards

The disease data used in the study were collected from eleven vineyards, all located in the Bordeaux area (Gironde, France). The vineyards are listed and described in Table 1. The cultivars were representative of the local appellation, with three known as being highly susceptible to esca (Cabernet-Sauvignon, Cabernet franc and Sauvignon Blanc), and two as being rather tolerant to it, Merlot Noir and Malbec (Dubos, 2002; Bruez *et al.*, 2013).

Vineyards with susceptible cultivars were selected for their high incidence of esca disease. The observations were carried out over three survey periods: 2004–2006, 2012–2014 and 2018–2021, representing a total of 27 vineyard*year situations and 10 years of field observations. The 2012–2014 survey was specifically designed to compare susceptible and tolerant cultivars. Vineyards with tolerant cultivars that were in close proximity to those with susceptible cultivars were selected.

2. Disease assessment, development of esca symptoms throughout summer and sanitary status

All the vineyards were monitored yearly to record the status of each originally planted individual vine, including missing replanted or retrained vines, as previously done in other studies (Lecomte *et al.,* 2012; Lecomte *et al.,* 2018). The survey consisted of regular monitoring during the vegetative season. Plants were at most observed twice a week, from early June (corresponding to the beginning of esca leaf symptom expression in this region) until mid-September or early October, after a complete onset and development of symptoms.

Data from a previous survey were used for the first period (2004–2006) (Lecomte *et al*., 2012). In the second survey period (2012–2014), foliar symptoms were monitored following the same procedure, as described by Lecomte *et al.* (2012). Briefly, three categories of visual leaf symptoms were defined: i) first-developing symptoms, corresponding to those also attributed to black dead arm disease (Larignon *et al*., 2001), with marginal or interveinal scorched zones and/or reddening on black cultivars (Figure 1, Leaf A) or pale green or yellowing zones on white cultivars (Figure 1, Leaves B and C), ii) leaves at an intermediate stage with both symptom profiles [i) and iii)] simultaneously

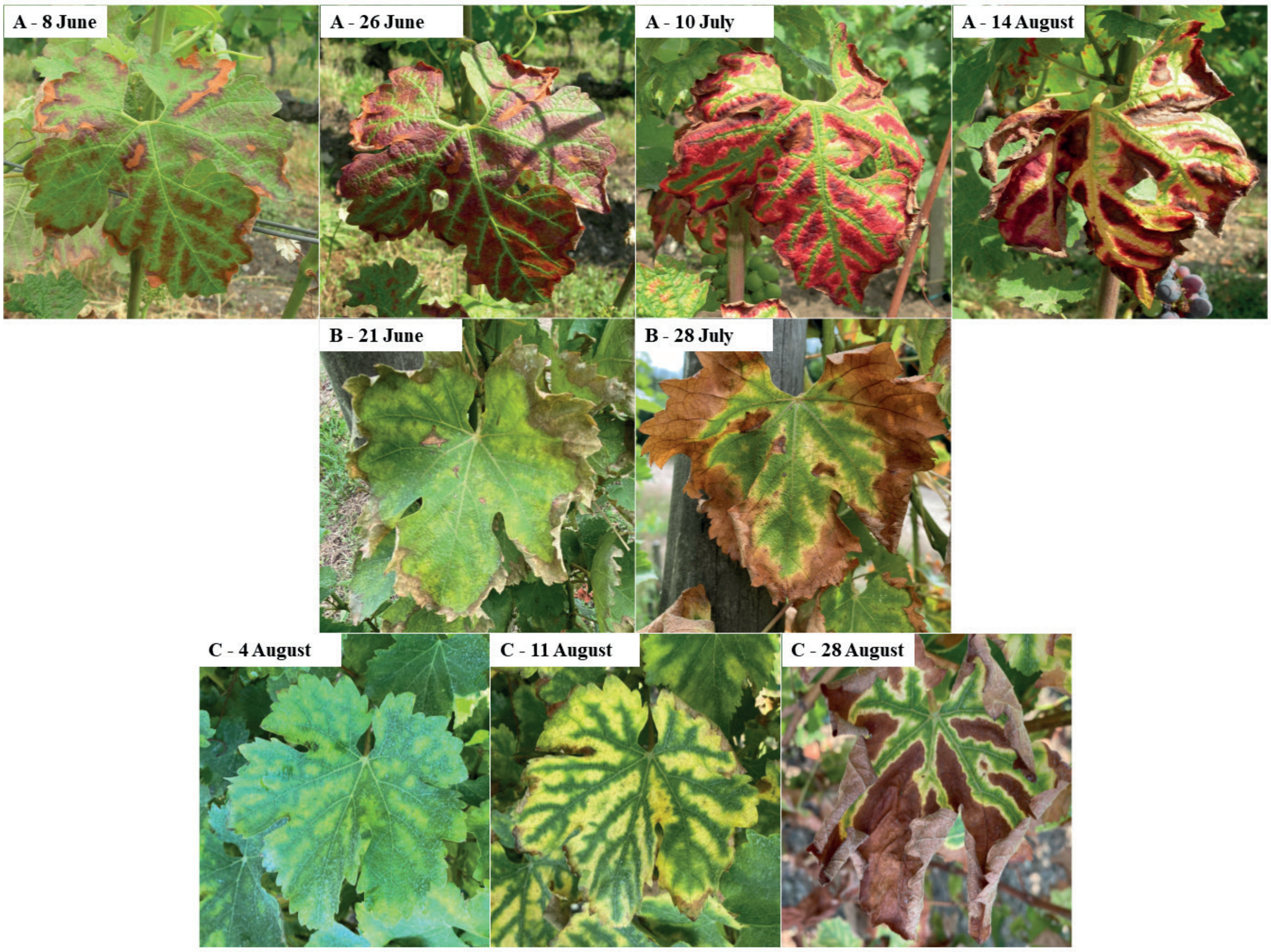

FIGURE 1. Summer development of three typical mild forms of an esca leaf symptom, showing on the same leaf, the different onset phases up to the typical tiger-striped aspect. Leaf A: black cultivar, Cabernet-Sauvignon (Pessac, 2012). Leafs B and C: white cultivar, Sauvignon blanc (Villenave d'Ornon, 2021).

observed on the same leaf; and iii) typical "tiger-stripe" esca symptoms in a final stage (Figure 1, Leaves A on August 14 and C on August 28). The severity of the symptoms was also recorded, as described in Lecomte *et al.* (2012) and Lecomte *et al.* (2018).

In the third period of the survey (2018–2021), the field observations of leaf symptoms were simplified by not distinguishing between the different stages of leaf development and by pooling all esca symptoms into one category; i.e., that of esca leaf-symptomatic vines.

In all the survey periods, the sanitary status of each vineyard was assessed by recording wood symptoms each year before the outbreak of the first foliar symptoms (Lecomte *et al.*, 2012). All trunk-affected vines (by esca and/or other grapevine trunk diseases (GTDs), referred to as unproductive, were categorised as either dead or missing vines, vines with only one living arm (out of a previous two), restored or retrained vines, or freshly replanted or young vines. The total number of unproductive vines was used to illustrate the sanitary status of each vineyard as a percentage of unproductive vines, determined by the ratio of the sum of unproductive vines to the total number of original vines that were planted (Table 1).

3. Examination of xylem stripes

The presence of longitudinal stripe(s) under the bark was checked, as exemplified in Figure 2a, by cutting and collecting a total of seventy-one vines from 2012 to 2014. This corresponds to 10 vines in July 2012 and 10 in July 2013 in vineyard CF CEN, 11 in July 2013 in vineyard SB VIL, 10 in April 2014 and 10 in July 2014 in vineyard CF CEN, and 10 in April 2014 and 10 in July in the vineyard SB VIL. The twenty vines collected in April were vines that had been leaf symptomatic the year before. All the other vines collected in July were vines showing recent esca foliar symptoms. Since the method was destructive, only severely leaf-affected vines were selected, and only two vineyards were prospected: one experimental vineyard, SB VIL, and one vineyard in the process of being uprooted, CF CEN. The minimum severity level was level 3 in the classification of Lecomte *et al.* (2012), Lecomte *et al.* (2018), Lecomte *et al.* (2022), corresponding to vines showing leaf fall, severe scorching or wilting on several canes of at least one cordon and apoplexy.

In addition, staining tests were used to colour the xylem vessels efficiently transporting the sap and to test the hydraulic functioning of the discolored stripes underneath the bark. In 2012, in the vineyard CS PES located at Pessac,

TABLE 1. Main characteristics of the eleven vine plots surveyed in Bordeaux area to examine the summer development of esca symptoms for three periods: 2004 to 2006, 2012 to 2014 and 2019 to 2021. Cultivars and years (2004–2006) used in a previous survey (Lecomte et al., 2012) are mentioned in italics. Cultivars that are considered tolerant to esca disease are mentioned in bold.

Vine-plot code[a]	*Farm name* Location Soil	Trellissing system[b]	Cultivar Rootstock	Year of planting	Year of survey	Observations		Phenology[d]	No. of surveyed vines[e]	% of wood-affected vines
						Period[c]	No.			
CS LUD	*Château La Lagune*	Espalier Guyot	*Cabernet-Sauvignon*	1981	*2004*	154-263	7	160-223	1	45.2
	Ludon-Médoc	Low form	5BB		*2005*	157-262	14	153-217	1	49.8
	Gravel	'Guyot double'								
CF LAT	*Le Grand Parc* Latresne Clay-limestone	Lyra	*Cabernet franc*	1987	*2004*	168-246	10	160-223	1,072	8.1
		High form	Fercal		*2005*	157-248	13	153-217	500	9.4
					2006	160-269	27	157-221	500	9.6
CS LAT		Espalier Guyot	Cabernet-Sauvignon	1997	2014	151-260	27	158-225	820	27.4
		Low form	Gravesac							
		'Guyot double'								
CS CEN	*Château Materre* Cénac Clay-limestone	Espalier Guyot High form 'Guyot simple'	*Cabernet-Sauvignon*	1988	*2005*	157-248	13	153-217	500	38.8
			3309C		*2006*	160-269	26	157-221	500	48.2
CF CEN			Cabernet franc	1988	2012	157-260	24	163-225	1,019	70.1
			3309C		2013	151-260	31	169-234	1,412[f]	78.8
MAL CEN			**Malbec**	1988	2013	151-260	31	169-234	675	40.0
			1103 Paulsen		2014	154-260	26	158-225	673	41.5
CS PES	*Pape Clément* Pessac Gravel-clay	Espalier Guyot Low form 'Guyot double'	*Cabernet Sauvignon*	1989	*2005*	157-262	14	153-217	500	9.4
			3310C		2012	154-260	25	163-225	1,059[f]	31.8
			CS PES		2013	151-260	31	169-234	1,059[f]	53.5
					2014	151-260	25	158-225	1,120[f]	34.3
MER PES			**Merlot**	1989	2013	151-260	31	169-234	872	10.1
			Unknown		2014	154-260	25	158-225	872	12.2
SB VIL	*Grande Ferrade* Villenave d'Ornon Gravel	Espalier Guyot Low form 'Guyot simple'	Sauvignon blanc	1991	2012	153-260	26	163-225	630	28.9
			101-14 MGT		2013	151-260	31	169-234	630	44.8
					2014	146-260	28	158-225	630	48.7
MER VIL			**Merlot**	1991	2013	151-260	31	169-234	630	0.6
			101-14 MGT		2014	154-260	26	158-225	630	0.6
SB COU	*Château Couhins*	Espalier Guyot	Sauvignon blanc	2001	2018	142-275	17	154-216	522	28.9
	Cadaujac	Low form	Fercal		2019	162-268	16	155-221	522	31.4
	Clay-limestone	'Guyot double'			2020	152-266	17	147-214	696	38.9
					2021	153-265	17	161-223	870	48.4

[a] Abbreviation of the vineyard name defined by using either the initials letters or the first three letters of the cultivar name and then the first three letters of the vineyard location; [b] Type of training system, height of the form (low: < = 50 cm; high > 70 cm) and type of pruning regime; [c] Julian dates of the first and the last summer field observations; [d] Phenology data reported here concerned the Julian dates corresponding to mid-flowering (BBCH 65) and mid-veraison (BBCH 85); [e] This number corresponds to the total number of vines that were originally planted, including all vines that were damaged after planting (dead, missing, re-planted or re-trained); [f] Rows surveyed that year were different for part from those previously surveyed.

FIGURE 2A. Example of xylem stripe visible under the bark of a leaf-symptomatic vine affected by esca disease (Picture: J.-M. Liminana).

five canes were cut and collected from Cabernet-Sauvignon symptomatic vines and five control canes from asymptomatic vines. The bases of the canes were immediately dipped in water, and the canes were transferred to the laboratory in wet plastic bags. The cuttings were refreshed underwater before an immediate transfer into a 0.5 % solution of safranin for at least 6 hours. The canes were recut approximately 10 cm above the base before being photographed. In 2019, in the 'Sauvignon Blanc' vineyard in Villenave d'Ornon, three symptomatic vines were cut just above the grafting point and immediately dipped in water. The cuttings were refreshed underwater before their quick transfer into an aqueous solution of phloxin (phloxin at 1 g/L, $CaCl_2$ at 0.11 g/L, Kcl at 0.75 g/L). The vines were kept upright and left in the vineyard for 6 hours before being peeled and photographed.

4. Modellisation of cumulative esca expression over time

All 27 available vineyard*year situations were used to model the appearance of esca leaf-symptomatic vines throughout the summer, but only 26 were illustrated: the vineyard*year situation CS PES 05, having a low number of leaf-symptomatic vines, was left out. Cumulative incidence rates were used as dependent variables and calculated as the percentages of the cumulative numbers of symptomatic vines observed at each observation date out of the total numbers of originally planted living vines (Table 2). These percentages were then transferred to scaled cumulative incidences between 0 and 1 and used to mathematically study the progress of esca expression.

Three different classical regression analyses were tested: linear, polynomial and logistic. These three regression models were chosen, because they are the most commonly used regression models and they provide a simple modeling approach that can be easily reused to analyse esca epidemiological data. These models were: first, a linear regression model Y = pr1 +pr2X; second, a third-order polynomial regression model $Y = pr1+pr2X+pr3X^2+pr4*X^3$; and third, a nonlinear logistic regression model Y = pr3/(1+Exp(-pr1-pr2X)) with its inverse equation X = ln (y)-npr1-ln (pr3-y))/pr2, where Y is the proportion (decimal number) of esca-symptomatic vines; X is time (Julian days) and:

- pr1 depends on the initial value (time 0),
- pr2 describes the rate of the speed in reaching the maximum number of symptomatic vines,
- and pr3 represents the maximum number of leaf-symptomatic vine increments (saturation).

Goodness of fit of regression models was assessed by using four correlation parameters: the coefficient of determination R-squared (R^2), the root of mean square error (RMSE), the Akaike Information Criterion (AIC), and the second-order Akaike Information Criterion (AICc), all provided by the statistical software XLSTAT (Addinsoft, 2023). Curves obtained from each vineyard*year situation allowed us to calculate the area under the disease progress curve (AUDPC) according to Madden *et al.* (2017). The latter were calculated from the adjusted values derived from the model equations for the period between the 140th and 260th Julian day (Table 2).

5. Phenological and meteorological data

Locally representative phenological data were obtained from an online network provided by the Bordeaux Faculty of Oenology (https://bordeauxraisins.fr/les-millesimes.html). The mid-flowering (BBCH phenological stage 65) and mid-veraison (BBCH stage 85) dates were recorded (Tables 1 and S1) and related to the periods of observations and of esca leaf symptom expression during the vegetative season. Mesoclimatic data originated from the INRAE Climatik network: https://agroclim.inrae.fr/climatik/. In the first two surveys, the standard meteorological station used (identification number: 3355003) was located at Villenave d'Ornon on the INRAE campus. It was selected for its proximity (less than 8 km away) to most of the vineyards except for the vineyard CS LUD in Ludon-Médoc.

TABLE 2. Data used to study the progress of esca leaf expression: total number of symptomatic vines (S) observed in each vineyard*year situation and related percentage, dates of first observed symptoms (S1), dates corresponding to 10 %, 50 % and 90 % provided by the regression logistic model and related sums of mean temperatures above 10 °C since 1st January, time between S10 % and S90 % and area under the disease progress curve (AUDPC) between Julian dates 140 and 260. Plot with tolerant cultivars are mentioned in bold.

Vineyard*year	No. surveyed living vines[a]	Symptomatic vines (S)		Date first observed symptom(s) S1[d]	Related ΣT > 10 °C for S1	Dates and related sums of temperature above 10 °C (ΣT > 10 °C) at three prevalence key-thresholds						No. of days between S10 % & S90 %	AUDPC 140-260
		No.[b]	%[c]			S10 %		S50 %		S90 %			
CS Lud 04	734	223	30.4	154 (June 2)	*296*	168 (June 16)	*459*	202 (July 20)	*790*	235 (Aug. 22)	*1197*	67	18.03
CF Lat 04	955	489	51.2	168 (June 16)	*459*	181 (June 29)	*594*	203 (July 21)	*802*	223 (Aug. 10)	*1064*	42	22.25
CS Lud 05	764	78	10.2	157 (June 6)	*427*	166 (June 15)	*517*	193 (July 12)	*858*	217 (Aug. 5)	*1153*	51	7.12
CF Lat 05	470	176	37.4	157 (June 6)	*427*	165 (June 14)	*510*	190 (July 9)	*816*	215 (Aug. 3)	*1130*	50	21.52
CS Pes 05	473	26	5.5	157 (June 6)	*427*	163 (June 12)	*490*	192 (July 11)	*842*	219 (Aug. 7)	*1176*	56	3.87
CS Cen 05	387	94	24.3	157 (June 6)	*427*	164 (June 13)	*501*	187 (July 6)	*791*	209 (July 28)	*1072*	45	14.74
CF Lat 06	467	281	60.2	160 (June 9)	*415*	171 (June 20)	*561*	193 (July 12)	*812*	216 (Aug. 4)	*1159*	45	45.26
CS Cen 06	394	142	36.0	160 (June 9)	*415*	169 (June 18)	*540*	190 (July 9)	*770*	215 (Aug. 3)	*1149*	46	27.52
CS Pes 12	916	230	25.1	154 (June 2)	*364*	166 (June 14)	*453*	189 (July 7)	*689*	213 (July 31)	*940*	47	17.54
CF Cen 12	421	75	17.8	157 (June 5)	*388*	163 (June 11)	*433*	194 (July 12)	*730*	225 (Aug. 12)	*1085*	62	11.64
SB Vil 12	576	400	69.4	153 (June 1)	*351*	160 (June 6)	*414*	180 (June 28)	*604*	200 (July 18)	*787*	40	54.97
CS Pes 13	702	129	18.4	157 (June 6)	*269*	189 (July 8)	*548*	223 (Aug. 11)	*1025*	236 (Aug. 24)	*1179*	47	7.99
CF Cen 13	506	85	16.8	179 (June 28)	*431*	195 (July 14)	*644*	219 (Aug. 7)	*983*	237 (Aug. 25)	*1186*	42	7.27
SB Vil 13	544	313	57.5	156 (June 5)	*258*	174 (June 23)	*396*	207 (July 26)	*832*	232 (Aug. 20)	*1130*	58	32.04
Mal Cen 13	520	49	9.4	183 (July 2)	*470*	201 (July 20)	*736*	222 (Aug. 10)	*1012*	239 (Aug. 27)	*1206*	38	3.76
Mer Pes 13	838	35	4.2	186 (July 5)	*501*	199 (July 18)	*704*	228 (Aug. 16)	*1084*	249 (Sept. 6)	*1315*	50	1.44
Mer Vil 13	626	13	2.1	197 (July 16)	*676*	213 (Aug. 1)	*914*	224 (Aug. 12)	*1037*	235 (Aug. 23)	*1169*	22	0.76
CS Lat 14	777	109	14.0	157 (June 6)	*391*	183 (July 2)	*690*	208 (July 27)	*972*	233 (Aug. 21)	*1217*	50	7.30
CS Pes 14	870	102	11.8	154 (June 3)	*365*	183 (July 2)	*690*	201 (July 20)	*881*	222 (Aug. 10)	*1127*	39	6.71
SB Vil 14	501	245	48.9	146 (May 26)	*319*	174 (June 23)	*600*	196 (July 15)	*816*	217 (Aug. 5)	*1069*	43	31.60
Mal Cen 14	513	32	6.2	198 (July 17)	*848*	198 (July 17)	*848*	213 (Aug. 1)	*1025*	231 (Aug. 19)	*1202*	34	2.86
Mer Pes 14	828	65	7.8	164 (June 13)	*480*	189 (July 8)	*751*	204 (Aug. 23)	*917*	228 (Aug. 16)	*1178*	39	4.10
Mer Vil 14	626	24	3.8	168 (June 17)	*522*	186 (July 5)	*725*	202 (July 21)	*892*	219 (Aug. 7)	*1092*	33	2.18
SB Cou 18	409	121	29.6	142 (May 22)	*281*	166 (June 15)	*493*	193 (July 12)	*829*	219 (Aug 7)	*1198*	53	24.03
SB Cou 19	405	164	40.5	162 (June 11)	*362*	179 (June 28)	*541*	207 (July 26)	*938*	237 (Aug 25)	*1269*	58	24.16
SB Cou 20	476	174	36.6	152 (May 31)	*481*	164 (June 12)	*561*	190 (July 8)	*809*	223 (Aug 10)	*1247*	59	23.92
SB Cou 21	506	240	47.4	153 (June 2)	*321*	178 (June 27)	*584*	198 (July 17)	*768*	219 (Aug 7)	*989*	41	28.19

[a] This number corresponds to the total number of original living vines that were able to show foliar symptoms, thus excluding all dead, lacking, retrained or replanted vines but including restored vines; [b] Total number of leaf-symptomatic vines observed along the summer season; [c] Percentages of leaf-symptomatic vines out of the total number of original living vines that were surveyed (as defined above). Cultivars that are considered tolerant to esca disease are mentioned in bold, their % of symptomatic vines and corresponding AUDPC as well; [d] The first date of observation allowed to record already-appeared symptoms in the few previous days. In most cases the first date of survey preceded the date of symptom appearance (Table 1).

In the third survey, meteorological data were collected from an available automatic station that was even closer (400 m) to Cadaujac (identification number: 33080002). Daily climate variables were mean temperatures, rainfall and potential evapotranspiration (Penman equation). For each year, sums of temperature above 10 °C and sums of rainfall were calculated as from 1st January on four key dates corresponding to the date of the first observed esca foliar symptom (S1) and dates to reach 10 % (S10 %), 50 % (S50 %) and 90 % (S90 %) threshold levels of symptomatic vines (Table 2).

6. Statistical analysis

For model calculations, all regression curves were processed using XLSTAT 2023 software distributed by the company Addinsoft, Paris (https://www.xlstat.com/). The statistical adjustment coefficients were provided by the software, and inverse equations of the logistic regression model were used to calculate the dates corresponding to S10 %, S50 % and S90 % thresholds of leaf-symptomatic vines (Table 2).

The software XLSTAT 2023 was also used to perform a principal component analysis (PCA) to study the relationships between the major characteristics of the 21 vineyard*year situations, disease features and climatic variables. The dataset comprised numerical scores attributed to the cultivars, soil types and trellising systems based on the literature and as described hereafter. Only the variable ‘cultivar’ was used as an explicative variable determined by a numerical score associated with each susceptibility level (tolerant = 1; susceptible = 2). The other explicative variables were calculated. They concerned the % of unproductive vines, the % of symptomatic vines, the AUDPC, the four key dates of symptom thresholds, S1, S10, S50 and S90, and their corresponding sums of rainfall or temperature (above 10 °C, initiated on 1st January). The variables ‘soil types’ and ‘trellising systems’, as well as the variable ‘time gap between S10 % and S90 %’, were used as supplementary quantitative variables. Scores for soil type were: 1 = gravel, 2 = gravel-clay and 3 = clay-limestone depending on their proportion of clay (Destrac-Irvine *et al*., 2005). The scores for trellising systems were: 1 = ‘lyra’, 2 = ‘guyot double’, and 3 = ‘guyot simple’ form according to Lecomte *et al.* (2018).

The software STATBOX PRO (Version 6.6, Grimmersoft Logiciels, Paris) was used to perform the variance analysis of 2013–2014 data (12 situations) to compare tolerant and susceptible cultivars. The variables were the temperature sums (above 10 °C as from 1st January) required to reach the four key thresholds, S1, S10, S50 and S90, as described above.

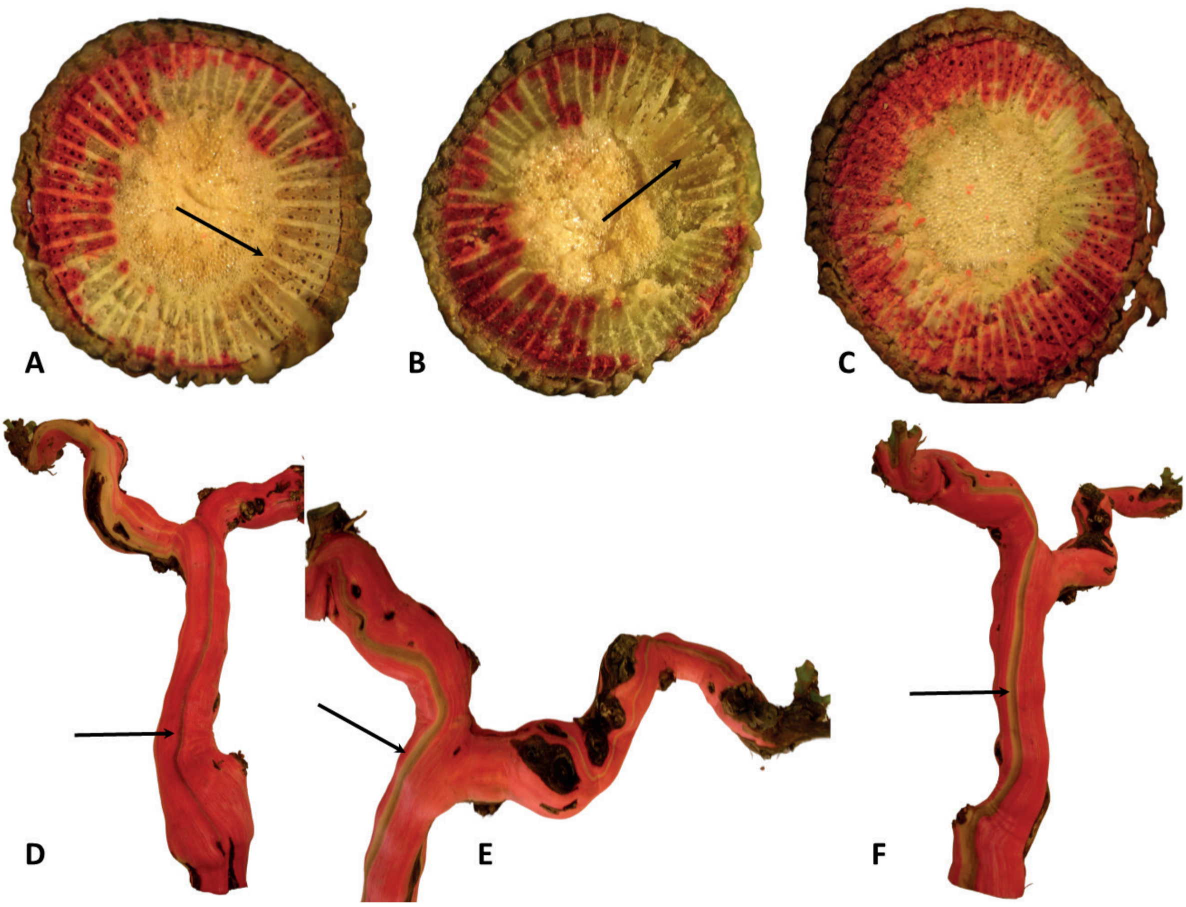

FIGURE 2B. Illustrations of the loss of hydraulic function after coloration of vessels that were still functional. Black arrows indicate the localization of either brown areas into the young canes (A and b) or brown-orangy stripes along the trunks (D to F). On top right, a control cane (C) with most functional sap routes and no stripe.

RESULTS

1. Vineyard observations, phenology and sanitary status of vineyards

Table 1 summarises the data and basic results from the three survey periods in this study: 8 vineyard*year situations in 2004–06, 15 in 2012–14 and 4 in 2018–21, representing a total of 20,913 surveyed vines. Tolerant cultivars (Merlot and Malbec in bold in Table 1) were represented by 6 situations, all monitored in 2013–14 (second survey), and susceptible cultivars (Cabernet-Sauvignon, Cabernet franc and Sauvignon blanc) corresponded to 21 situations distributed over the three surveys.

Symptom monitoring most often covered a minimum 15-week period from early June to mid-September (as from Julian dates of approx. 150 to 260). The first vineyard visual symptom assessments began at approximately mid-flowering in late spring, on dates between 21 May 2018 and 16 June 2004 (Julian dates 142 and 168 respectively). The last observations occurred mostly on dates in September after mid-veraison and close to or during the harvest period. These varied between September 2 (in 2004) and October 2 (in 2018) (Julian dates 246 to 275 respectively), because observations were continued at some sites to ensure that new symptomatic vines did not appear again. Except for in the first survey, throughout the period from late spring to late summer, the number of observations (at least once a week) per vineyard*year situation varied from 16 to 31.

The percentages of trunk-affected vines ranged markedly from 0.6 % to 78 %, mostly reaching more than 30 % for the susceptible cultivars (14 situations out of 21). The Merlot cultivar displayed percentages of trunk-affected vines of less than 12 % at Pessac and less than 1 % at Villenave d'Ornon. The Malbec cultivar showed approximately 40 % trunk-affected vines, a level similar to that of many susceptible cultivars.

2. Esca foliar symptoms and development of the xylem stripes

As illustrated in Figure 1, symptoms evolved according to a continuum of development from one category to the next, resulting in typical esca tiger-striped symptoms or apoplectic forms at the end of the season. As a first category, all vines showing recent symptoms displayed symptoms corresponding to those also attributed to black dead arm disease (Larignon *et al*., 2021). Then, as they aged, they exhibited an intermediate stage (second category) before typical esca symptoms, as exemplified by Figures S1a and S1b. The summer observations showed that the threshold of 90 % of the total number of symptomatic vines recorded during the season was generally reached before September (Table 2).

Regarding the presence of longitudinal stripe(s) under the bark, all leaf-symptomatic vines examined between 2012 and 2014 exhibited this typical kind of symptom from canes exhibiting leaf symptoms up to the upper part of trunks. Concerning the possible associated loss of hydraulic function, the five canes collected from symptomatic vines in 2012 and the three diseased vines collected in 2019 showed all vessel tissues to no longer be functional (Figure 2b).

3. Differential esca expression and early appearance in tolerant and susceptible varieties

The percentages of symptomatic vines, out of the total number of living vines (Table 2), ranged from 2.1 % to 69.4 %. Tolerant cultivars generally displayed the lowest percentages (2.1 % to 9.4 %), while susceptible cultivars, except for one situation (CS Pes 05, 5.5 %), showed much variable and higher percentages (10.2 % to 69.4 %). The first observed symptoms (S1) appeared from the Julian dates 142 to 198, representing a long time gap of 56 days, with a mean date for the whole survey of 162 (Table 2). On susceptible cultivars, the first symptoms appeared between Julian dates 142 and 179 (time interval = 37 days; average = 157), corresponding to the beginning of June and to the mid-flowering period or just after. Equivalent dates for tolerant cultivars were often later and ranged between 164 and 198 (six situations, time interval = 36), with an average date of 183.

4. Modelling of esca expression over time

Three steps characterised the temporal development of the typical esca foliar symptoms (Figures S1a, S1b, 3a and 3b). The first step, often shorter for susceptible cultivars, included a slow and progressive appearance of vines exhibiting the typical symptoms. The second step was characterised by a longer period and a fast increase in the number of vines expressing the symptoms. The third step corresponded to a gradual decrease in new symptomatic vines. Almost all symptomatic vines had appeared by the Julian date 250; i.e., by early September.

Compared with a linear or a third-order polynomial model, the logistic regression curves were the best for representing and modelling esca foliar symptom development over time using cumulative incidence rates of esca-symptomatic vines (Figures 3a and 3b). In the 27 situations studied, the typical sigmoidal symptom-development profile was well-represented by the logistic regression model. According to the corresponding equations and statistical adjustment coefficients (Table 3), the coefficients of determination R^2 were all systematically superior to 0.98 and greater than those obtained with either the linear model or the polynomial model. Thus, a high-quality prediction of the logistic model was demonstrated. Moreover, when comparing the different models tested, from the AIC values it was clear that the linear model was not the best one (Table 3). When further comparing the polynomial and logistic models, the two Akaike criteria, notably the second-order Akaike Information Criterion, showed that the logistic model was better than the polynomial model (no exception by using the AICc index) (Table 3). Because the numbers of symptomatic vines were generally low for tolerant cultivars, the values of the equation parameter pr3 were also generally low for those cultivars (< 0.1); pr2 values varied between 0.064 and 0.186, and pr1 values were often higher than 16.7 for tolerant cultivars. Even

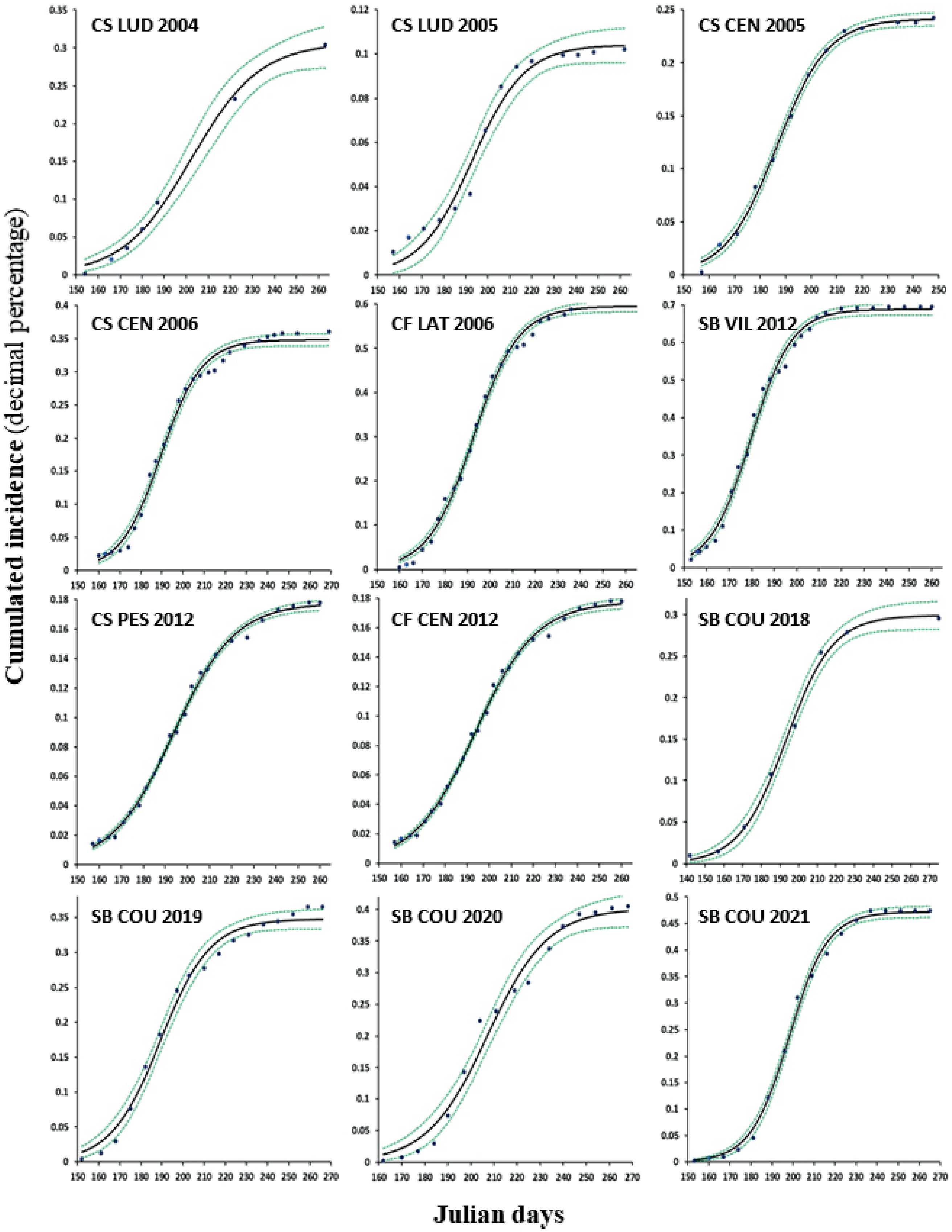

FIGURE 3A. Logistic regression curves of the dynamic of esca foliar expression observed between 2004 and 2021 in twelve vineyard situations. Lines with green dashes indicates the confidence intervals at *P* = 0.05.

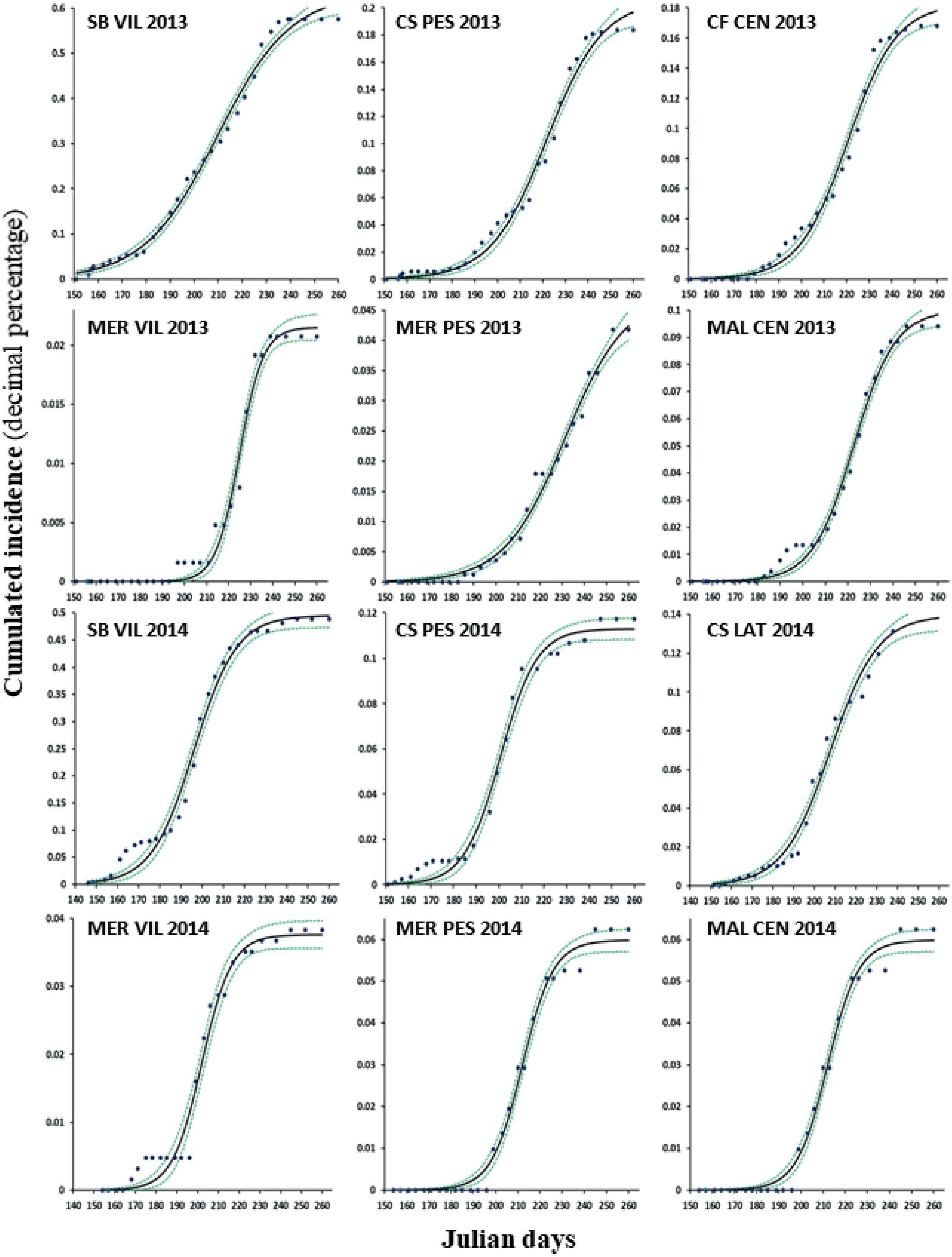

FIGURE 3B. Logistic regression curves of the dynamic of esca foliar expression observed in 2013 and 2014 in 12 vineyard situations with susceptible and tolerant cultivars alternatively. Lines with green dashes indicates the confidence intervals at *P* = 0.05.

TABLE 3. Regression model equations tested from the disease data recorded in the 27 'vineyard*year' situations examined in Gironde between 2004 and 2021 and their robustness assessed according to three statistical adjustment coefficients. Plots with cultivars considered tolerant to esca disease are mentioned in bold. Best coefficient values per 'vineyard*year' situation are indicated in bold.

Vineyard*Year	Linear model			Polynomial model order 3				Non linear logistic model				
	y = pr1+pr2x			y = pr1+pr2x+pr3x²+pr4x³				y = pr3/(1+Exp(-pr1-pr2x))				
	R^2 [a]	RMSE[b]	AIC[c]	R^2	RMSE	AIC	AICc[d]	Equation per 'vineyard*year'	R^2	RMSE	AIC	AICc
CS Lud 04	0.975	0.020	-53	**0.999**	**0.005**	**-71**	-11	y = 0.307/(1+Exp(13.033-0.064x))	0.995	0.010	-60	**-40**
CS Lud 05	0.871	0.014	-118	0.954	0.009	-126	-119	y = 0.104/(1+Exp(16.123-0.084x))	**0.976**	**0.007**	**-136**	**-132**
CF Lat 04	0.940	0.053	-57	0.988	0.027	-67	-52	y = 0.523/(1+Exp(20.213-0.100x))	**0.991**	**0.022**	**-72**	**-64**
CF Lat 05	0.925	0.039	-82	0.993	0.013	-107	-99	y = 0.378/(1+Exp(16.304-0.086x))	**0.996**	**0.009**	**-119**	**-114**
CF Lat 06	0.882	0.078	-136	0.982	0.032	-181	-179	y = 0.595/(1+Exp(19.248-0.100x))	**0.995**	**0.016**	**-218**	**-217**
CS Lat 14	0.932	0.014	-229	0.984	0.007	-262	-260	y = 0.140/(1+Exp(18.240-0.088x))	**0.988**	**0.006**	**-271**	**-270**
CS Pes 05	0.904	0.006	-139	0.983	0.003	-158	-151	y = 0.056/(1+Exp(14.463-0.075x))	**0.988**	**0.002**	**-165**	**-161**
CS Pes 12	0.885	0.033	-169	0.981	0.014	-208	-205	y = 0.248/(1+Exp(18.266-0.097x))	**0.993**	**0.008**	**-236**	**-234**
CS Pes 13	0.885	0.024	-230	0.971	0.012	-267	-265	y = 0.206/(1+Exp(17.790-0.080x))	**0.988**	**0.008**	**-296**	**-295**
CS Pes 14	0.911	0.014	-210	0.968	0.009	-230	-227	y = 0.113/(1+Exp(24.993-0.125x))	**0.989**	**0.005**	**-257**	**-255**
Mer Pes 13	0.850	0.005	-322	0.986	**0.002**	-389	-387	y = 0.048/(1+Exp(16.766-0.072x))	**0.989**	**0.002**	**-397**	**-396**
Mer Pes 14	0.903	0.010	-228	0.967	0.006	-249	-246	y = 0.072/(1+Exp(30.701-0.151x))	**0.986**	**0.004**	**-271**	**-269**
CS Cen 05	0.883	0.032	-88	0.988	0.011	-111	-103	y = 0.241/(1+Exp(18.577-0.100x))	**0.997**	**0.005**	**-131**	**-126**
CS Cen 06	0.858	0.049	-155	0.970	0.023	-190	-187	y = 0.348/(1+Exp(19.509-0.103x))	**0.992**	**0.012**	**-225**	**-223**
CF Cen 12	0.941	0.015	-200	0.990	0.006	-237	-234	y = 0.178/(1+Exp(13.897-0.072x))	**0.997**	**0.003**	**-269**	**-266**
CF Cen 13	0.883	0.023	-233	0.971	0.012	-270	-268	y = 0.183/(1+Exp(20.213-0.092x))	**0.989**	**0.007**	**-302**	**-300**
Mal Cen 13	0.850	0.014	-262	0.961	0.007	-298	-296	y = 0.100/(1+Exp(24.161-0.109x))	**0.992**	**0.003**	**-347**	**-345**
Mal Cen 14	0.869	0.009	-242	0.969	0.005	-274	-271	y = 0.060/(1+Exp(30.192-0.142x))	**0.991**	**0.003**	**-306**	**-304**
SB Vil 12	0.803	0.117	-110	0.980	0.039	-163	-160	y = 0.688/(1+Exp(19.748-0.110x))	**0.994**	**0.021**	**-197**	**-195**
SB Vil 13	0.956	0.046	-189	**0.991**	0.022	-231	-229	y = 0.633/(1+Exp(13.687-0.065x))	**0.991**	**0.021**	**-235**	**-234**
SB Vil 14	0.903	0.061	-155	0.968	0.036	-180	-178	y = 0.495/(1+Exp(19.500-0.099x))	**0.987**	**0.024**	**-204**	**-203**
Mer Vil 13	0.759	0.004	-338	0.917	0.003	-364	-362	y = 0.022/(1+Exp(41.836-0.186x))	**0.988**	**0.002**	**-425**	**-423**
Mer Vil 14	0.886	0.005	-270	0.954	0.004	-288	-285	y = 0.038/(1+Exp(28.189-0.139x))	**0.981**	**0.002**	**-311**	**-309**
SB Cou 18	0.869	0.047	-47	0.994	0.013	-65	-35	y = 0.299/(1+Exp(15.408-0.080x))	**0.997**	**0.008**	**-74**	**-60**
SB Cou 19	0.952	0.036	-105	**0.986**	**0.021**	**-118**	-112	y = 0.401/(1+Exp(15.785-0.076x))	0.984	0.022	-117	**-114**
SB Cou 20	0.904	0.042	-106	0.981	0.020	-127	-122	y = 0.348/(1+Exp(16.009-0.085x))	**0.989**	**0.015**	**-138**	**-134**
SB Cou 21	0.905	0.064	-92	0.982	0.030	-114	-109	y = 0.472/(1+Exp(21.612-0.109x))	**0.997**	**0.011**	**-147**	**-144**

[a] Linear regression coefficient; [b] Root of the Mean of the Squares Error; [c] Akaike Information Criterion; [d] Second-order Akaike Information Criterion

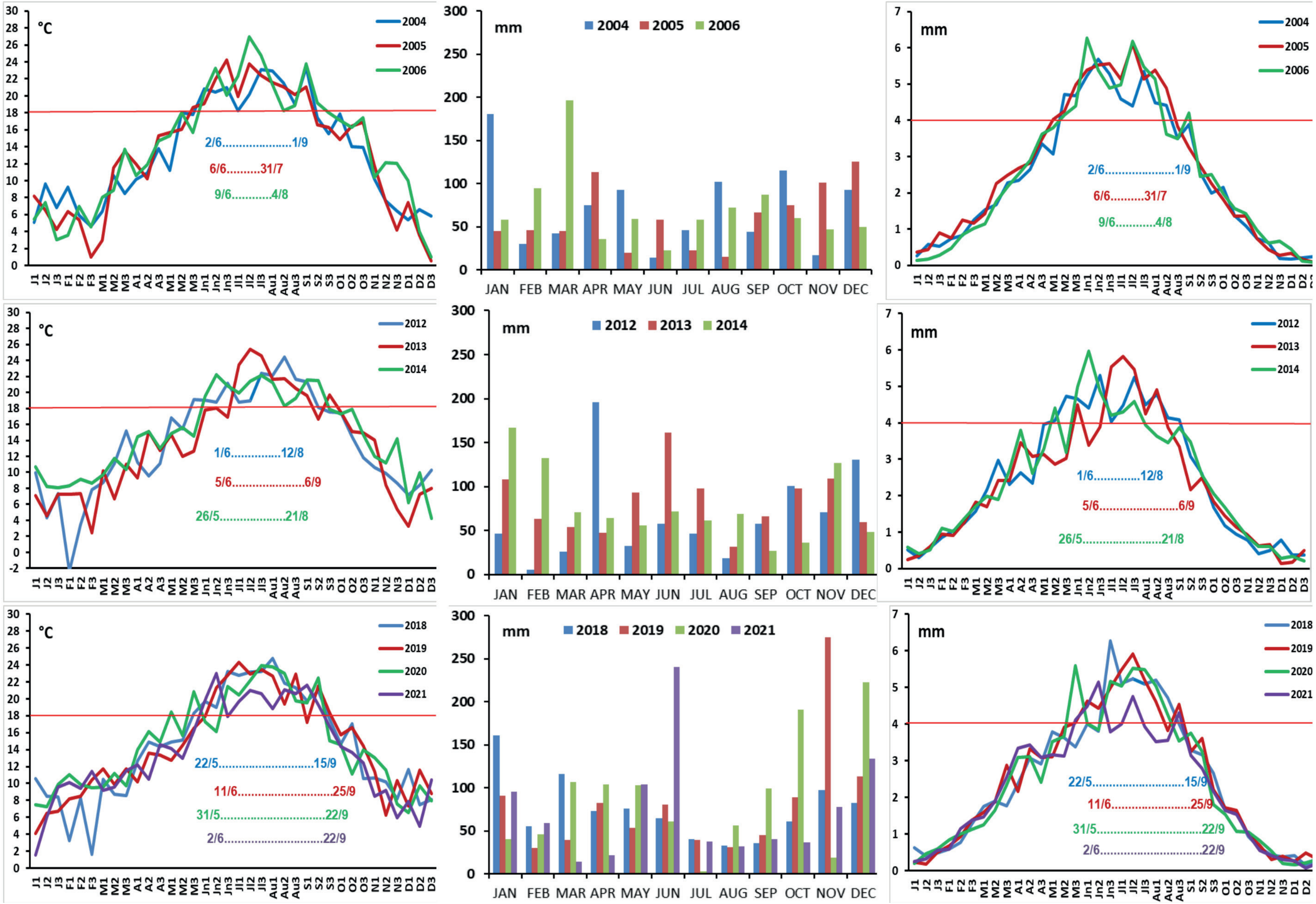

FIGURE 4. Representations of the main climatic parameters recorded for the three studied periods: mean of average temperatures in °C per decade (left), monthly rainfall in mm (central) and mean estimated evaporation in mm per decade using Penman equation (right).

TABLE 4. Results of the variance analysis comparing susceptible and tolerant cultivars for the years 2013 and 2014 using as variables : the sums of temperature above 10 °C (ΣT) reached at 4 symptom appearance key-thresholds, corresponding to S1, S10 %, S50 % and S90 %, the rainfall amounts (ΣR) for the same key-tresholds, the length of time between dates corresponding to S10% and to S90% for each 'cultivar x year' situation.

Variable	Factors	F test	Pr > F*	Mean ΣT All cultivars	Mean Σ	Newmans-Keuls test
ΣT S1	**Cultivar****	**11.23**	**0.009**	461 °C	**Susceptible: 339 Tolerant: 583**	**S**
	Year	0.54	0.490		2013: 434 2014: 487	NS
	Interaction	0.04	0.842			NS
ΣT S10 %	**Cultivar**	**11.62**	**0.009**	687 °C	**Susceptible : 595 Tolerant: 780**	**S**
	Year	1.23	0.299		2013: 657 2014: 717	NS
	Interaction	1.68	0.229			NS
ΣT S50 %	Cultivar	3.07	0.115	952 °C	Susceptible: 918 Tolerant: 994	NS
	Year	3.23	0.107		2013: 996 2014: 917	NS
	Interaction	0.24	0.640			NS
ΣT S90 %	Cultivar	1.38	0.274	1175 °C	Susceptible: 1,151 Tolerant: 1,194	NS
	Year	1.92	0.201		2013: 1,198 2014: 1,148	NS
	Interaction	0.39	0.552			NS
ΣR S1	**Cultivar**	**6.51**	**0.033**	494 mm	**Susceptible: 455 Tolerant: 532**	**S**
	Year	1.55	0.247		2013: 475 Tolerant: 532	NS
	Interaction	1.40	0.270			NS
ΣR S10 %	Cultivar	3.30	0.104	556 mm	Susceptible: 542 Tolerant: 571	NS
	Year	1.56	0.246		2013: 546 2014: 566	NS
	Interaction	0.09	0.760			NS
ΣR S50 %	Cultivar	2.06	0.187	628.5 mm	Susceptible: 624 Tolerant: 633	NS
	Year	**17.06**	**0.003**		**2013: 642 2014: 615**	**S**
	Interaction	0.05	0.823			NS
ΣR S90 %	Cultivar	0.57	0.047	653 mm	Susceptible: 650 Tolerant: 657	NS
	Year	0.1	0.756		2013: 652 2014: 655	NS
	Interaction	0.05	0.819			NS
Time S10 % to S90 %	Cultivar	4.33	0.069	41 days	Susceptible: 46.5 Tolerant: 36	NS
	Year	0.39	0.553		2013: 43 2014: 39	NS
	Interaction	0.13	0.724			NS

* Probability to reject the null hypothesis (no factor effect) at 5 % significance level.

** Factors that had a significant effect are indicated in bold.

if the graph profiles were similar between vineyard*year situations, there was a clear time lag in the beginning of the curves between susceptible and tolerant cultivars in the onset of the first esca foliar symptoms.

5. Comparison of tolerant and susceptible varieties in terms of key dates, AUDPC values and temperature sums

As indicated in Table 2, following the logistic model, key dates corresponding to 10 % of the total number of symptomatic vines (S10 %), ranged between 160 and 213 (time interval = 53 days; mean date = 178). For susceptible cultivars, these dates varied from 160 to 195 (interval = 35 d; mean = 173), and for tolerant cultivars, they varied from 186 to 213 (interval = 27 d; mean = 198). For S50 %, the corresponding dates ranged between 180 and 228 (interval = 48 d; mean = 202). For susceptible cultivars, the dates varied from 180 to 219 (interval = 39; mean = 198), and for tolerant cultivars, they varied from 202 to 228 (interval = 26 d; mean = 215). Finally, for S90 % the dates ranged between 200 and 249 (gap = 49 d; mean = 225). For susceptible cultivars the dates varied from 200 to 239 (interval = 39 d; mean = 222), and for tolerant cultivars they varied from 219 to 249 (interval = 30 d; mean = 234). Thus, the following trend was clearly shown: tolerant cultivars mostly developed symptoms later than susceptible cultivars.

For the time interval between S10 % and S90 %, the overall mean was 47 days (Table 2). Interestingly, for the susceptible cultivars (mean of 50), most of the time intervals were equal to or longer than 40 days (20 situations out of 21). For tolerant cultivars (average of 36 days), the corresponding duration was mostly lower than 40 days (5 situations out of 6).

AUDPC values during the period 140-260 varied logically in a similar way to the percentages of symptomatic vines. They ranged between 2.1 % and 54.9 %. Tolerant cultivars generally displayed the lowest values ranging from 2.1 % to 4.1 %, while susceptible cultivars showed higher values ranging from 6.7 to 54.9 (except for one situation: CS PES 05 with 3.8).

Crucial relationships were demonstrated by further statistically analysing the symptom threshold dates in relation to cultivar susceptibility and climatic variables, including temperature and rainfall (Table 4, two-factor ANOVA with cultivar and year main effects). The data used were those recorded in 2013 and 2014, because these were the only seasons allowing a strict comparison of susceptible *versus* tolerant cultivars. There was a significant difference (P = 0.05) between susceptible and tolerant cultivars for three variables: ‘Sum of temperature to reach S1’, ‘Sum of temperature to reach S10 %’ and ‘Sum of rainfall to reach S1’. Susceptible cultivars required significantly less temperature sums on average than tolerant cultivars to reach dates corresponding to S1 and S10 % and less cumulative rain on average to exhibit the first symptoms. No significant cultivar effect was shown by considering the other variables, in particular the ‘Sum of temperature to reach S50 %’, the ‘Sum of temperature to reach S90 %’ and the ‘Time between S10 and 90 %’.

6. Relationships between esca symptom expression and some climatic parameters

Three key mesoclimatic parameters were analysed in the three survey periods (Figure 4). Rainfall amounts per decade were irregularly distributed over the year and more variable from one year to the next. However, the means of average temperatures and the mean estimated evaporation per decade noticeably showed that the periods of symptom expression regularly corresponded to the warmest and driest time of each year. Accordingly, on the date corresponding to the first symptoms observed (S1), the sums of temperatures (above 10 °C since 1st January) ranged between 258 °C and 848 °C, resulting in a large difference (590 °C) between extreme situations. For susceptible cultivars, the corresponding sums of temperatures varied from 258 °C to 481 °C (gap = 223 °C), and for tolerant cultivars from 470 °C to 848 °C (gap = 418). Similarly, a large difference (590 °C) between extreme situations was demonstrated using the S10 % threshold of 10 % of symptomatic vines (temperature sums ranging between 258 °C and 848 °C). For susceptible cultivars, the temperature sums varied from 258 °C to 481 °C (gap = 223 °C), and for tolerant cultivars, the sums were all higher, varying from 470 °C to 848 °C (gap = 418). For S50 %, the values ranged between 604 °C and 1084 °C, with a large difference (480 °C) between extreme situations. For susceptible cultivars, the sums varied from 604 °C to 983 °C (gap = 379 °C), and for tolerant cultivars, they varied from 892 °C to 1084 °C (gap = 192). For the S90 % stage, the sums ranged between 787 °C and 1315 °C; i.e., a difference of 528 °C between extreme situations. For susceptible cultivars they varied from 787 °C to 1269 °C (gap = 482 °C), and for tolerant cultivars from 1092 °C to 1315 °C (gap = 223 °C). Thus, the S10 % threshold best discriminated susceptible and tolerant cultivars.

7. Relationships between esca symptom expression, vineyard characteristics and climatic features

Using data issued from Tables 1 and 2, the relationships between vineyard characteristics, level of disease expression and the temperature and rainfall variables were assessed by principal component analysis (PCA). The first two PCA axes accounted for nearly three-quarters of the total variance (i.e., 72.5 %), with the first key PCA axis accounting for 59.1 % of the total variance (Figure 5). The first important result showed that the cultivars were clearly differentiated by their susceptibility to the disease; i.e., all the tolerant cultivars were located and grouped in the top right-hand side of the biplot, not including any susceptible cultivars. Thus, the first PCA axis showed a clear contrast between the susceptible cultivars on the left-hand side with negative coordinates, notably the highly susceptible Sauvignon blanc “SB” (contribution to the axis of 17.7 %), and the tolerant ones, Merlot “Mer” and Malbec “Mal”, on the right-hand side with positive coordinates (contribution to the axis of 50.3 %). This contrast also resulted from different temperature regimes, showing that tolerant cultivars were characterised by late dates of early disease expression, notably at the S10 threshold.

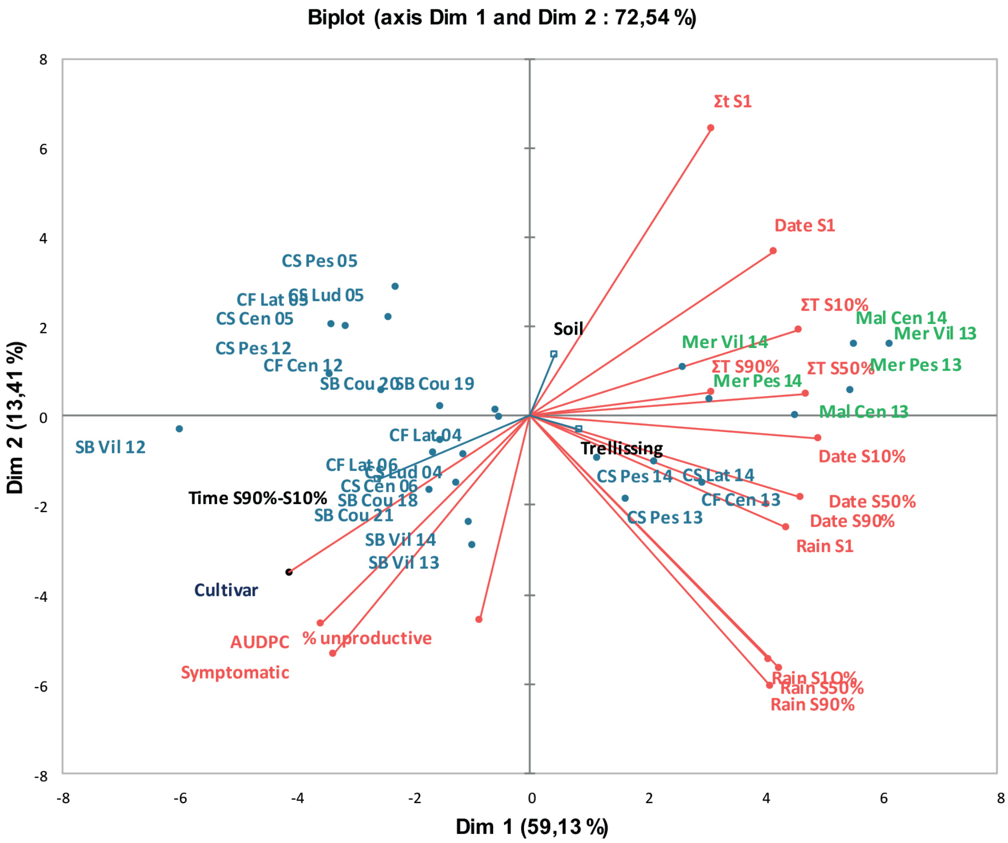

FIGURE 5. Biplot of Principal Component Analysis to illustrate the relationship between the main characteristics of the 27 'vineyard*year' situations, disease data and climatic parameters. Active variables were: cultivar, three disease criteria (% of unproductive vines, % of symptomatic vines and AUDPC), dates of observation of symptoms corresponding the tresholds S1, S10 %, S50 % and S90 % and their corresponding sums of temperature above 10 °C and those of rainfall amount, since 1st January. Soil, trellissing and time gap between S10 % and S90 % dates were used as supplementary quantitative variables and year as supplementary qualitative variable.

This was clearly shown by the contribution of the corresponding variables to the first axis: "Date-S10 %", "Date-S50 %", "ΣT-S10 %" and "ΣT-S50 %" with contributions of 9.5 %, 8.4 %, 8.3 % and 8.7 % respectively. The second main PCA axis (13.4 % of the total variance) mostly represented - on its positive side - the sum of temperature required to observe the 1st symptoms; i.e., variable "ΣT-S1" with a contribution of 16.3 % to the axis. Moreover, the positive side of axis 2 mostly corresponded to the plots monitored in 2005 (contribution 37.1 %). The main active negative variables were: i) the rain variables (Rain-S10 %, Rain-S50 % and Rain-S90 % with cumulative contributions to the axis reaching 38.7 %), and ii) three high-disease expression variables with cumulative contributions of 27.9 %; i.e., AUDPC, Symptomatic incidence and percentage of unproductive vines. Overall, as shown by their direct symmetric opposition, there was a clear antagonism between a high sum of temperature reached to observe the 1st symptoms "ΣT1" and the three key high-expression disease variables; i.e., AUDPC, Symptomatic incidence and Cultivar susceptibility (Figure 5); the Pearson's corresponding correlation coefficients were significant, reaching -0.469, -0.496 and -0.713, respectively (dF = 25, significant at $P = 0.05$ for AUDPC and at $P = 0.01$ for the two other disease variables). To exhibit the 1st typical esca foliar symptoms, susceptible cultivars required lower cumulative temperature sums than tolerant cultivars. Symptoms in susceptible cultivars were therefore observed earlier in the season.

DISCUSSION

In this work, we investigated the temporal development of esca leaf symptoms over time in Bordeaux vineyards as a follow-up to the results presented by Lecomte *et al.* in 2012. There were four main goals: first, we described the progressive onset of leaf symptoms during summer and confirmed that initial foliar symptoms are indistinguishable from those attributed to black dead arm; second, we further examined the presence of xylem longitudinal vessel discolorations and explored the relationship with the putative loss of hydraulic function; third, we modelled the sigmoidal profiles of the cumulative incidence of esca leaf symptoms as previously observed (Lecomte *et al.*, 2012); and last, we explored the relationships between the progressive appearance of esca leaf symptoms in early summer and temperature, a potential major triggering climatic factor of esca pathogenesis.

We used disease data originating from 27 Bordeaux vineyard*year situations based on three survey periods. Two variables were used: the percentage of vines showing typical foliar symptoms and the percentage of trunk-affected vines. Both variables generally showed disease levels consistent with the known susceptibility of the cultivars (Dubos, 2002). More surprising was the Malbec cultivar, which had an unexpectedly high percentage of unproductive vines (Table 1). This may be due to the quality of pruning or to a particular response of this cultivar, which shows fewer foliar symptoms and more wood-dieback symptoms than other cultivars (this is also sometimes noticeable in other supposedly tolerant grape varieties, notably the Merlot noir cultivar when severely pruned (Lecomte *et al*., 2018).

1. Foliar symptoms and the development of xylem stripes

Our previous study showed that the temporal development of esca leaf symptoms is closely associated with typical xylem discolorations under the bark of diseased vines (Lecomte *et al*., 2012). This finding, which highlights the key role of the vascular system, was recently reinforced by research conducted on the impact of esca on vine transpiration (Ouadi *et al*., 2019; Ouadi *et al*., 2021; Bortolami *et al*., 2021b) and on the integrity of xylem vessels and hydraulic functioning (Bortolami *et al*., 2019; Bortolami *et al*., 2021a; Bortolami *et al*., 2023). The new surveys, presented here and conducted from 2012 to 2014 in 15 vineyard*year situations, largely confirmed the evolutive development pattern over time, as previously published (Lecomte *et al*., 2012). The temporal evolution of the visual aspect of esca leaf symptoms in three phases further confirmed that the typical tiger-striped leaf scorch symptom is an advanced symptom and that earlier symptoms, sometimes attributed to black dead arm, cannot be dissociated from esca. Furthermore, the two more recent and original surveys in this study, 2012–2014 and 2018–2021, also confirmed that the first symptoms can appear in late May or early June and that most symptoms develop before mid-August, corroborating other findings obtained under various conditions (Surico *et al.,* 2000; Marchi *et al*., 2006; Lecomte *et al.,* 2012).

The longitudinal xylem stripe symptom, already described in the past (Arnaud and Arnaud, 1931), was observed under the bark in all the examined esca-diseased vines, demonstrating that this symptom is generic to esca. Staining of the xylem sap flow indicates that the xylem vessels of this discoloured stripe were no longer functional. The origin of such symptoms may be associated with occlusions and the presence of gums or tyloses, as observed in young (Larignon, 2010) or old vines (Pouzoulet *et al.*, unpublished data; Bortolami *et al*., 2021a). A preliminary hypothesis regarding the origin of this xylem stripe was based on a sudden sap disruption in a warm period of water shortage (Lecomte *et al.*, 2012). This hypothesis may be supported by grapevine sap flow disruption in response to esca (Ouadi *et al.*, 2019; Ouadi *et al.*, 2021). Accordingly, it has been recently shown that esca leaf symptom development leads to decreased transpiration at the plant level (Bortolami *et al*., 2021b) and decreased stem hydraulic conductance due to the occlusion of xylem vessels (Bortolami *et al.*, 2021a). However, more research is needed to better understand the mechanisms underlying the formation of this nonfunctional xylem stripe and the relationship with fungal development within wood tissues, notably just under the bark or nearby.

2. Modelling of esca expression over time

In all the surveyed vineyard plots, the cumulative incidence of esca foliar symptoms followed a clear, progressive and typical sigmoidal pattern. The evolution of esca incidence over time was monitored very closely on each vine on a weekly (or fortnightly) basis, particularly to record the onset of foliar symptoms. This rate of observation was sufficient to ensure a robust dataset for developing and testing an adapted modelling approach. Three models were tested and compared, showing that the logistic model provided the best fit for the increase in esca incidence during the season. Logistic models have already been used for grapevine trunk diseases, such as *Eutypa* dieback (Kaplan *et al.,* 2016 based on Duthie *et al.*, 1991), or to show that white rot, one of the internal necroses of the trunk, is the best predictor of the chronic form of esca (Guérin-Dubrana *et al.*, 2012). Here, we fitted the logistic model to the progression of esca foliar expression over time. This enabled us to demonstrate an overall regular progression pattern, irrespective of plot, year and maximum incidence in the vineyard plot under consideration. Logistic regression models are a suitable approach for accurately describing polycyclic diseases that involve multiple disease cycles occurring within a single growing season (Madden *et al*., 2017; Nutter, 1997). However, in the epidemiological case of esca, the infection process is most often considered to involve a few primo-infection steps targeting the grapevine pruning wounds at the end of winter and/or the beginning of spring (Gramaje *et al*., 2018). Because secondary cycles during the season have not been clearly demonstrated in the esca-grapevine pathosystem to date, further investigation should be carried out to determine their presence. However, there is currently no substantiated evidence of a spreading-contagion process in the vineyard from one vine to the next (Li *et al.*, 2017). Therefore, it is necessary to further investigate the epidemiological mechanisms to explain why a logistic model fits the esca incidence progression over time within the vineyard during the growing season. We can hypothesise that a common biological triggering mechanism, such as the progressive rise in temperature during the first half of the year, contributes to explaining such a clear pattern of progression in symptom expression. The differences between equation parameters under various circumstances could be further investigated; it would also be interesting to analyse deviations between specific observed data points and corresponding model curves, and attempt to correlate them with specific environmental variables. For example, in the SB COU 20 situation, a warm and dry period (after Julian Day 210) was correlated with a slowing down of emerging symptoms, extended drought being a significant factor that influences esca expression, as demonstrated by Bortolami *et al*. (2021b) on the same cultivar. For additional information, the role of temperature was further examined using thermal data as an alternative X-axis for the models in the Couhins vineyard. Very similar patterns were obtained, as shown in Figure S2, confirming the close relationship of esca expression with temperature and a clear deviation for the SB COU 20 situation during the warm and dry period.

3. Comparison between tolerant and susceptible cultivars and interaction with temperature

Our modelling approach and statistical analyses revealed significant differences between tolerant and susceptible cultivars. The results of the multidimensional PCA can be summarised as follows: the tolerant cultivars i) were less affected by the disease, as anticipated, ii) they developed symptoms at a later stage than the susceptible ones, iii) they required a significantly greater amount of temperature sums, on average, to reach key dates corresponding to S1 and S10 %, and iv) they also required a higher cumulative rainfall, on average, to exhibit the onset of symptoms.

The present study clearly and systematically demonstrates that the period of symptom expression corresponded to the first half of summer. This finding further supports our main hypothesis that the intensification of symptoms is a regular biological process, as previously suggested (Lecomte *et al*., 2012). Similarly, Marchi *et al.* (2006) observed most esca symptoms to occur in July, but they could not identify any clear climatic pattern to explain this. However, during this period, there is a regular increase in mean temperatures with less frequent rainfall events and higher evapotranspiration. The role of temperature in fungal growth, particularly for plant pathogens, is well-documented in the literature (Fischer and Peighami-Ashnaei, 2019; Songy *et al.*, 2019; Claverie *et al.*, 2020). Thus, temperature may act as a trigger for symptom appearance by accelerating microbial colonisation or activity, leading to an unbalanced microbial situation in the host plant. This hypothesis is in agreement with two recent articles: i) Serra *et al.* in 2018 demonstrated that a temperature rise from 50 % sprouting until June resulted in a greater number of new symptomatic plants, as evidenced by monthly foliar symptom evolution, and ii) Ouadi *et al.* (2021) observed an earlier expression of esca symptoms with higher summer temperatures in 2018 than in 2017 in a vineyard located near Bordeaux. While temperature may play a significant role in triggering symptoms and increasing disease incidence and severity by directly influencing fungal activity (Chaloner *et al*., 2021), it can also become a limiting factor when too high. For example, in *Botrytis cinerea*, symptoms are reduced when temperatures exceed the threshold of 30 °C, as high temperatures inhibit mycelial growth (Ciliberti *et al*., 2015; Calvo-Garrido *et al*., 2021). Leaf symptoms can also be inhibited by severe drought periods (Bortolami *et al*., 2021b). Thermal and water stresses have also been identified as driving factors of grapevine trunk disease development (Songy *et al*., 2019). As an illustration, out of a total of 2,820 surveyed vines in another Sauvignon blanc Couhins vineyard (2020), 526 vines (18.6 %) showed no leaf or wood symptoms, including 247 showing dehydrated leaves (likely due to drought or heat). The year after (2021), among these 247 vines, 105 vines (43 %) were leaf symptomatic, while among the 279 vines that did not exhibit symptoms of dehydration, only 55 (20 %) were affected by the disease. This sequence of symptomatic status suggests that the same factor (e.g., an abiotic stress) could sometimes be a trigger or inhibitor depending on the climatic conditions and plant status, although the physiological status of the plant needs to be assessed to test whether abiotic stresses are actually occurring in the vineyard. While the role of rainfall and the underlying role of water availability is not within the main scope of this study, no clear relationships were found to explain the emergence of esca symptoms with regard to the graphs illustrating the rainfall or with data used in the PCA. However, according to past literature, rainfall seems to interact more with the incidence level of symptomatic vines than with the onset of symptoms. To conclude, this study allowed us to demonstrate differences in the date of the first symptom observation between susceptible and tolerant cultivars.

4. Toward a comprehensive model of the onset of esca symptoms

The etiology of esca disease is still a matter of debate, particularly regarding the mechanism(s) responsible for the appearance of foliar symptoms. These mechanisms are still not fully understood and remain controversial. Claverie *et al.* (2020) reformulated two main hypotheses regarding the cause of leaf symptom appearance: i) the impact of phytotoxic compounds or toxins, as previously reviewed by Andolfi *et al*. (2011), and ii) a disruption of sap flow, as suggested by Lecomte *et al*. (2012), resulting in hydraulic failure, as explored by Bortolami *et al.* (2019), Bortolami *et al.* (2021a), and Bortolami *et al.* (2023).

The results of our study suggest that each year, at the start of the season, there may be a reservoir of a particular number of diseased vines that are on the brink of a critical unbalanced situation. As temperatures rise over the summer, these vines may begin to display visible leaf symptoms. In other words, this suggests that the disease is likely already present at the beginning of the season and becomes externally apparent as the season progresses. Throughout its life, a vine may be subjected to various biotic or abiotic stresses, including infection by trunk pathogens. These stresses can lead to the formation of necrosis, notably in the wood and under the bark, which can vary in shape, volume, and appearance depending on the type of wood-inhabiting fungi present. When necroses occur in the outer regions of the xylem, they reduce the amount of functional tissue available for transporting water and nutrients. The development of pathogenic fungi is influenced by factors such as temperature and water availability, which can also be impacted by the cultural techniques used. Thus, each vine has a unique history in relation to trunk pathogens. During early summer, the substantial increase in daily mean temperatures may thus promote fungal activity in the woody sections of the vine, as well as increase the evaporative demand. In mature vines, the highest concentration of fungal activity is likely located at the top of trunks or at the base of cordons (Bénétreau *et al*., 2019), where longitudinal stripes are frequently observed (Lecomte *et al*., 2012). After the initial stages of disease expression, an unbalanced state may arise, characterised by a defense response of the plant leading to a decrease in water availability and hydraulic failure of only certain xylem vessels, presumably in the vicinity of the most active fungal site. This could occur just before the formation of the brown stripe and the onset of leaf symptoms. In the Bordeaux region, this hypothesis is supported by previous observations of apoplectic or severely esca-affected vines in May or June, particularly during periods of unusually high temperatures. Surico *et al.* (2000) has also reported such a phenomenon in other viticultural regions experiencing water stress and high temperatures. However, future research is needed to fully understand the mechanisms underlying the formation of the brown stripe.

While the toxins hypothesis has been studied *in vitro* for a long time (Andolfi *et al*., 2011), the investigation of the hydraulic failure hypothesis is more recent. Leaf symptom onset has been recently associated with the disruption of vessel integrity and the presence of tyloses and gels that occlude the vessels of symptomatic leaves (Bortolami *et al*., 2019; Bortolami *et al*., 2023) and shoots of the year (Bortolami *et al*., 2021a). This also suggests that such mechanisms of nongazeous embolism could account for brown stripe development. Vessel occlusions can occur either naturally with xylem aging or in response to various biotic or abiotic stresses in the sapwood of perennial organs (De Micco *et al.*, 2016), while in leaves, occlusions seem specific to esca disease (Bortolami *et al*., 2023). Although it is commonly accepted that embolism precedes vessel occlusion (Brodersen *et al*., 2010), air embolism was not observed during esca (Bortolami *et al*., 2021a). Moreover, the reduction in xylem water transport with the onset of esca symptoms did not affect the plant water status (Bortolami *et al.*, 2021b). Thus, it should be further investigated which key events may precede vessel occlusion. This should be addressed under the particular multi-stress conditions of increased fungal activity due to higher temperatures and when water availability decreases (and/or following an increased evaporative demand). Accordingly, by focusing more on apoplectic esca, such a facies could originate from interactions between high vapour pressure deficit (VPD), associated with increased temperatures, and the significant volume of nonfunctional necrotic wood in which active fungal development may also play a role.

In keeping with previous findings (Ouadi *et al*., 2019), Bortolami *et al.* (2021a) hypothesised that a signal (toxins and/or elicitors) passing through the xylem network and accumulating in the leaves could stimulate tylosis formation and lead to hydraulic failure. In addition, hormonal biosynthesis of ethylene or auxin, as one possible stress response, could also be a key factor causing such occlusions (De Micco *et al*., 2016). Plant hormonal signals could also explain leaf fall during esca, which is partial in the case of a severe esca symptom, limited to one or a few canes, or is total in the case of apoplexy.

Esca disease has been described either as a complex unique disease, i.e., a syndrome (Lecomte *et al.*, 2012), or a complex of diseases (Mugnai *et al.*, 1999; Andolfi *et al*., 2011). Surico (2009) has also defined the esca complex as a complex of five syndromes. Among them, white rot was almost always associated with the basidiomycete *Fomitiporia mediterranea*, but was not linked with tiger-striped foliar symptoms. However, recent studies tend to clearly demonstrate that white rot is not only related to apoplexy but is also involved in the development of typical esca leaf stripe symptoms. F. *mediterranea* development (Maher *et al*., 2012; Ouadi *et al*., 2019) or abundance (Del Frari *et al*., 2021) in grapevine wood has been clearly associated with esca foliar symptoms or a sudden collapse in summer. In addition, recent studies on the curettage technique have confirmed that the removal of white rot reduces the onset of esca foliar symptoms, thus pointing to the very likely role of *Fomitiporia mediterranea* in their appearance (Cholet *et al*., 2021; Pacetti *et al*., 2021; Lecomte *et al.*, 2022). Therefore, the five different syndromes should be considered as different symptoms of the same disease, that is esca, as described by Ravaz (1909), Viala (1926) and Arnaud & Arnaud (1931). Esca is a peculiar pathological syndrome resulting from inner necrosis developing from wounds following grafting or pruning. The different altered wood configurations can disturb the hydraulic system in young or older vines (showing longitudinal stripes) and can lead to consecutive typical leaf symptoms. More attention should now be paid to all possible impacts on inner wood leading to hydraulic failure in relationship with fungal activity and water availability in a warm season, as suggested by the present study. Additionally, we propose the logistic model for use as a descriptive, analytical and potentially predictive tool for examining esca expression in the vineyard.

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