Phytopathologia Mediterranea (2013) 52, 2, 276−288

RESEARCH PAPER

Statistical analysis of grapevine mortality associated with esca or Eutypa dieback foliar expression

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Summary. Esca and Eutypa dieback are two major wood diseases of grapevine in France. Their widespread distribution in vineyards leads to vine decline and to a loss in productivity. However, little is known either about the temporal dynamics of these diseases at plant level, and equally, the relationships between foliar expression of the diseases and vine death is relatively unknown too. To investigate this last question, the vines of six vineyards cv. Cabernet Sauvignon in the Bordeaux region were surveyed, by recording foliar symptoms, dead arms and dead plants from 2004 to 2010. In 2008, 2009 and 2010, approximately five percent of the asymptomatic vines died but the percentage of dead vines which had previously expressed esca foliar symptoms was higher, and varied between vineyards. A logistic regression model was used to determine the previous years of symptomatic expression associated with vine mortality. The mortality of esca is always associated with the foliar symptom expression of the year preceding vine death. One or two other earlier years of expression frequently represented additional risk factors. The Eutypa dieback symptom was also a risk factor of death, superior or equal to that of esca. The study of the internal necroses of vines expressing esca or Eutypa dieback is discussed in the light of these statistical results.

Key words: grapevine, trunk diseases, epidemiology, logistic regression.

Introduction

Esca and Eutypa dieback, considered worldwide as two major wood diseases of grapevine (*Vitis vinifera* L.), cause a decrease in productivity and a reduction in vine lifetime (Munkvold *et al*., 1994; Mugnai *et al*., 1999; Creaser and Wicks, 2001). In France, following the banning of sodium arsenite, a large survey aimed at assessing the evolution of grapevine trunk diseases over a six-year period (2003 to 2008) showed that about 11% of vineyard areas were unproductive, due to vine decline and death (Kobès *et al*., 2005) associated with the presence of esca and Eutypa dieback. Both diseases were characterized by development of these pathogens in the woody tissues of the vines and by external symptoms on leaves and berries.

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Eutypa dieback, also called dying-arm disease, is caused by an ascomycetous fungus, *Eutypa lata* Tul. & C. Tul., which penetrates into the plant via pruning wounds and then infects the woody tissues (Moller and Kasimatis, 1978). Several years after infection, typical wedge-shaped necroses can be observed in cross-sectioned cordons and trunks, which are associated with external cankers. These symptoms may also be associated with death of the spurs, cordons or entire grapevines. *Eutypa lata* produces various phytotoxins, some of which are transported by the transpiration stream towards the aerial part of the vine where the foliar symptoms can be seen (Moly-

ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

neux *et al*., 2002; Andolfi *et al*., 2011). After bud break, symptoms of stunted new shoots, which often have small, deformed chlorotic leaves and small fruit clusters can be observed.

The etiology of esca is more complex than that of Eutypa dieback. The term esca is generally used to designate a disease complex (Mugnai *et al*., 1999; Graniti *et al*., 2000; Surico *et al*., 2006). Presence of the vascular pathogens: *Phaeomoniella chlamydospora* (W. Gams, Crous & M.J. Wingf. & L. Mugnai) Crous & W. Gams, *Phaeoacremonium* spp. (mainly *P. aleophilum* W. Gams, Crous, M.J. Wingf. & L. Mugnai) (Surico, 2009) can cause typical foliar tiger stripe symptoms, recently termed "grape leaf stripe disease (GLSD)". The wood decay aspect of esca which is visible as a white rot inside the infected trunks and cordons is caused by the basidiomycete fungus, *Fomitiporia mediterranea* M. Fischer, in European countries (Fischer, 2006). In adult vines expressing typical tiger stripe symptoms on leaves, white rot in the wood tissues of the vines, is often associated with various other necroses (Mugnai *et al*., 1999; Surico *et al*., 2006; Calzarano and Di Marco, 2007; White *et al*., 2011). When GLSD is associated with wood decay, it represents the chronic form of "esca proper" (Graniti *et al*., 2000). Apoplexy, the sudden wilt of vines that occurs in summer, is generally considered as the severe (or acute) form of esca and terminates in partial or total vine death (Mugnai *et al*., 1999; Surico *et al*., 2006). This symptom can also be associated with both wedge-shaped necroses in the trunk or arm, and with a large proportion of non-functional tissue (Luque *et al*. *al*., 2012). Apoplexy is assumed to be caused, at least in part, by toxins of fungal origin (Letousey *et al*., 2010). In this paper, the term chronic esca to indicate GLSD or esca proper are used, without checking all the vines showing symptoms for the presence of white rot. The term of acute esca (apoplexy form) is used for the total wilt of vines recorded in summer. The term Eutypa dieback was used when the typical foliar symptoms were recorded, including stunting.

Even if trunk wood diseases constitute a major threat for vineyards, numerous key questions concerning the epidemiology of these diseases still remain to be investigated. For instance, there is no answers to relatively basic questions such as: how long can the infected vines survive? How is it possible to increase vine lifetime? It is, therefore, essential to carry out studies on the temporal dynamics of trunk wood diseases, including the ultimate stage, that of vine death. The two main obstacles to that come from the slow and cryptic development of internal necroses in the wood tissues, and the erratic foliar expression of the diseases. Discontinuity in symptom expression is a characteristic of both esca and Eutypa dieback. For esca, vineyard surveys over several years have shown that the symptoms fluctuate from one year to another, and that plants that express foliar symptoms one year do not necessarily express those symptoms the following year (Hewitt, 1957; Mugnai *et al*., 1999; Surico *et al*., 2000; Redondo *et al*., 2001; Di Marco *et al*., 2011; Marchi *et al*., 2006). For instance, Marchi *et al*. (2006) showed that 27% of vines that had previously expressed esca foliar symptoms did not express symptoms in any of the following five years. Several authors also reported discontinuity in foliar expression for Eutypa dieback (Le Gall, 1992; Creaser and Wicks, 2001; Dumot *et al*., 2004; Sosnowski *et al*., 2007). Even though the reasons for such fluctuations have not been fully established, several hypotheses can be advanced. Péros *et al.* (1999) suggested that variations in symptoms observed in the vineyard could be partly explained by the great genetic diversity in *E. lata* population added to its interaction with fungal communities in wood necroses. Foliar symptom fluctuation could also be attributed to particular interactions between toxic metabolites produced by the involved fungi, and/or to abiotic factors such as rainfall, temperature and grapevine management. According to Surico *et al*. (2000), Marchi *et al*. (2006) and Sosnowski *et al*. (2007), climatic factors presumably influence foliar expression for esca and Eutypa dieback. Marchi *et al*. (2006) showed that the number of vines that displayed foliar symptoms of esca throughout a growing season was directly related to the rainfall in May–June or in summer, depending on the vineyards. Surico *et al*. (2000) observed that cool and rainy summers favoured the expression of chronic esca, but that acute esca *i.e.* apoplexy, was more common during the hot summer period.

Currently, there are very few statistical studies about the relationships between esca or *Eutypa* dieback and mortality, either at field or plant level (Fussler *et al*., 2008; Péros *et al*., 2008). For six years, Surico *et al*. (2000) monitored esca and dead vines in vineyards planted with different cultivars. Before vine death, the plant varied in its annual frequency of symptom expression, which led the authors to conclude that the occurrence of external symptoms in a given year was very difficult to predict. Although the vine dying process is complex, one way of studying trunk diseases could be to focus on the temporal relationship between esca or Eutypa dieback foliar symptoms and vine mortality at plant level. In this case, individual vine-death can be viewed as a rare, stochastic event, and the logistic models presented here offer particularly appropriate tools to determine the risk of vine death, since they are based on the occurrence of the symptoms over a period of several years preceding vine death. This approach could be a first step in encouraging the development of trunk disease-predicting models in order to improve short- and long-term vineyard management strategies. To achieve this, Stefanini *et al*. (2000), used data recorded in a field in Italy and applied a logistic multimonial model to evaluate the conditional probability that a vine would show symptoms or die, based on the presence of symptoms in the previous year and on the presence of symptoms in the neighbourhood.

The objectives of the present paper are to study the relationships between esca foliar or Eutypa dieback foliar expression and vine mortality in six vineyards, planted with cv. Cabernet Sauvignon, located in different areas of the Bordeaux region. Individual vine recordings over seven years were used to determine the percentage of vine death, whether the vines had previously expressed foliar symptoms or not. As a first step in our modelling approach, logistic regression analysis is used to determine the probability of death based on previously occurring foliar symptoms. A sample of vines that had previously expressed esca or Eutypa dieback was used to verify and to quantify internal necroses. The statistical results are discussed in the light of necrosis analysis.

Materials and methods

Vineyard survey

Esca, Eutypa dieback and mortality were monitored in six different vineyards, belonging to several owners in the Bordeaux region, for seven consecutive years, from 2004 to 2010. The vineyards were selected since they did not show symptoms of the other main causes of dieback: viral diseases and *Armillaria* diseases. All vineyards were planted with the cultivar Cabernet Sauvignon (*Vitis vinifera* L) between 1986 and 1989 and were trained in accordance with the Guyot method. The vines were grafted onto various rootstocks (101-14 Mgt, 3309 Couderc, and SO4) depending on the vineyard (Table 1). In May/June of each year, about 2,000 contiguous vines from each vineyard were individually surveyed for typical foliar symptoms of Eutypa dieback, including the stunting of new shoots with chlorotic, small and cupped leaves, and vines or cordons with no vegetation were recorded as dead vines. At the end of August, the vines expressing chronic esca and those expressing acute esca symptoms were also recorded.

Data management and analysis

For each year, observations from each individual recorded plant were classified into eight categories: chronic esca, acute esca, Eutypa dieback, chronic or acute esca and Eutypa dieback, dead cordon, dead vine, asymptomatic adult vine and asymptomatic young vine. The data were used to calculate the annual disease incidence of chronic esca, defined as the ratio between the number of vines exhibiting chronic esca symptoms in a given year, and the number of vines alive the year before in the vineyard, multi-

Table 1. Characteristics of the six monitored vineyards located in the Bordeaux region (France).

Vineyard	Region	Commune	Year of planting	Rootstock	Vine number
1	Pessac Léognan	Martillac	1989	101-14	2000
2	Graves	Castres	1989	3309 Couderc	2366
3	Pessac Léognan	Gradignan	1989	3309 Couderc	2040
4	Libournais	Galgon	1987	3309 Couderc	1993
5	Graves	Beautiran	1989	-	2028
6	Entre-deux-mers	Espiet	1986	SO4	2050

plied by 100. The annual incidence of other disorders was also calculated: acute esca, Eutypa dieback, and newly dead vines (cordons or entire vines). The Spearman correlation test was applied to study the strength of relationship between esca incidence in year n and death incidence in year n+1.

The percentage of dead vines (cordons or entire vines) was calculated in each of six vineyards, for 2008, 2009 and 2010, using numbers of vines that had previously expressed esca symptoms (chronic or acute) at least once, from 2004 to 2007, to 2008 and to 2009, respectively. A similar procedure was applied for previously asymptomatic vines for the same respective periods. The same method was also used to determine the percentage of dead vines for vines expressing Eutypa dieback symptoms in each of three vineyards that showed the highest level of Eutypa dieback incidence. Logistic regression analysis was used to determine vine death risk in each vineyard for each of the three years 2008, 2009 and 2010 on the basis of previously occurring foliar symptoms (esca and Eutypa dieback) for the four previous years, from 2004 to 2007, from 2005 to 2008, from 2006 to 2009, respectively. As esca and Eutypa dieback foliar symptoms were always recorded on adult vines, the data set contained observations that were restricted to adult plants, in order to homogenize the set. To determine vine death risk in a vineyard in 2008, according to esca symptoms recorded in the preceding years, the observations were classified annually during the 2004-2007 period, as either asymptomatic or esca symptomatic (chronic or acute form). Other observations, including symptomatic Eutypa dieback or dead vines for any year before death, were removed from the data set. For 2008, each observation was classified as either of two categories, living or dead (cordon or entire vine). In order to compare results for each of the three years (2008, 2009 2010), death risk was modeled using the data set which included symptoms for the four preceding years. The data set was prepared for each year of death and each vineyard. Data management was also carried out to determine vine death risk according to Eutypa dieback symptoms in the three vineyards showing the highest level of Eutypa dieback incidence.

For each year and each vineyard, multiple binary logistic regression was used to identify the years in which foliar symptoms were significantly associated with vine death. This form of regression is used when the dependent variable Y is dichotomous (usually Y encodes the presence/absence of an event such as vine death in the given year in this case) and the independent variables (also called covariates) $X_1,\ldots,X_k$ are of any type (Hosmer *et al*., 1989). The method proposes a model for the probability of occurrence of an event occurring given the values of the covariates:

$$P(Y=1|x_1\cdots x_k)=\frac{e^{(\beta_0+\beta_1 x_1+\cdots+\beta_k x_k)}}{1+e^{(\beta_0+\beta_1 x_1+\cdots+\beta_k x_k)}}.$$

Coefficient β_0 is the intercept and e^{β_0} estimates the probability of death without symptoms when there is globally weak prevalence (in this case, with approximately 5% of dead vines). The impact of the covariate x_j on the probability of occurrence is measured through e^{β_j}, which is an odds ratio (OR). In the context of weak global incidence, the OR could be interpreted as a relative risk. In our study, when an OR for the variable "Symptom in a given year X (S0X)" was equal to two, this meant that the probability of death occurring was double that of vines without such symptoms. An OR significantly greater than one indicated that symptoms in a given year represented a risk factor. The interaction between years was also tested. Interaction terms between the statistically significant variables ''Symptom in a given year'' were added to the main effects model and were retained if they were statistically significant ($P<0.05$). For a significant interaction between the variable S0X (variable « Symptom in the year X ») and S0Y (variable « Symptom in the year Y »), the corresponding OR was the product of the ORs of S0X, S0y and S0XxS0Y. In order to select the covariates and then their interactions, a manual backward method was used.

Necrosis analysis

Twenty scions of vines expressing chronic esca symptoms or Eutypa dieback foliar symptoms in 2004 were collected in February 2005 from the surveyed vineyards, in order to quantify cases of internal necrosis. The trunks and cordons were cut into sections and analysed in accordance with the method of Maher *et al*. (2012). Three types of necrosis were considered: (i) sectorial brown necrosis, (ii) total necrosis, that included sectorial and central necroses, and (iii) white rot, characterised by yellow or white soft rot. The relative area covered by these necroses was calculated by dividing the necrotic area by the total area of the wood section. The altered perimeter, corresponding to the external perimeter of sectorial necrosis or total necrosis, was also measured. The relative perimeter length was calculated by dividing the altered perimeter by the total perimeter of the wood section. The Wilcoxon rank test was used to compare internal necroses in the cordon alone and in the whole scion between symptomatic and asymptomatic grapevines and between the two types of symptoms (esca and Eutypa dieback). That non-parametric statistical test is used to compare two independent samples. All statistical analyses were performed with R 2.8.1 software (R Development Core Team, 2008).

Results

The annual incidence of disease symptoms and dead vines over time

The annual esca (acute and chronic form) incidence varied from 1.4% to 16.8% depending on the vineyard and the year (Figure 1). The chronic form, observed more frequently than the acute form, reached its highest level in 2007 in all six vineyards. For most of the vineyards, the lowest incidence for both forms was observed in 2005. The incidence of Eutypa dieback also varied between 0 and 14.4% according to the vineyard and the year. It was highest in vineyards 4, 5 and 6, reaching greater values than those of esca in vineyard 6. The incidence of Eutypa dieback, like that of esca, was also highest in 2007, except for vineyard 5. The proportion of plants expressing both types of symptoms for a given year was very low, varying from 0 to 2.3% (data not shown). The incidence of partially or totally dead vines differed according to the vineyard and the year, not always showing proportionally similar numbers of vines expressing esca or Eutypa dieback. The incidence of dead vines was usually less than that of vines expressing esca and / or Eutypa dieback, but increased during the last three years (2008, 2009 and 2010) in vineyards 4 and 6. For all six vineyards, the incidence of newly dead vines increased in the last three years, with a peak in vine mortality in 2008 being preceded by a peak of esca symptoms in 2007. The Spearman rank correlation test indicated a significant correlation ($P<0.01$) for the incidence of esca symptoms in year n and the incidence of dead vines in year n+1 ($r = 0.78$). The percentage of partially dead vines (vines with one dead cordon) was higher than that of totally dead vine.

Mortality according to symptom expression

Using data from the seven-year individual vine recordings, the incidence of dead vines and dead cordons in 2008, 2009 and 2010 was determined according to whether or not esca symptoms had been expressed at least once in the previous four, five or six years respectively (Figure 2). The average incidence of dead vines among the vines that had previously expressed esca varied from 7.5 to 41.5% (Figure 2A), in the different vineyards. The lowest percentage was found for vineyard 1 and the highest for vineyard 4. For all the vineyards, the percentage of dead vines in 2008 that had previously expressed esca was higher than or equal to, death in the other years.

Although the dead vine incidence among previously asymptomatic vines was lower than that among vines previously expressing esca symptoms, it reached 9% of the asymptomatic vines in vineyard 6 in 2010 (Figure 2B). Similar variations were observed between vineyards; when the dead vine incidence for vines that had previously expressed symptoms was high, the asymptomatic dead vine incidence was also high.

In three of the six vineyards with a high incidence of Eutypa dieback, the percentage of dead vines among the vines expressing Eutypa dieback symptoms varied between 9.4 and 45.5%, depending on the vineyard (Figure 3A), with the lowest incidence of dead vines being found in vineyard 5. The percentage of dead vines in 2008 that had previously expressed Eutypa dieback symptoms was higher than in the other years. The dead vine incidence among previously asymptomatic vines was lower than that among vines previously expressing Eutypa dieback symptoms and, just like esca, it also reached 9% of the asymptomatic vines in vineyard 6 in 2010 (Figure 3B). Vineyard 5 was characterized by low incidence of dead vines among vines that had previously expressed foliar symptoms and by low dead vine incidence for asymptomatic vines.

Previous years of symptomatic expression associated with mortality

Esca foliar symptoms

The multiple binary logistic regression model was used to select, from among the four variables for a previous year of esca foliar expression (acute or chronic), those associated with mortality in 2008,

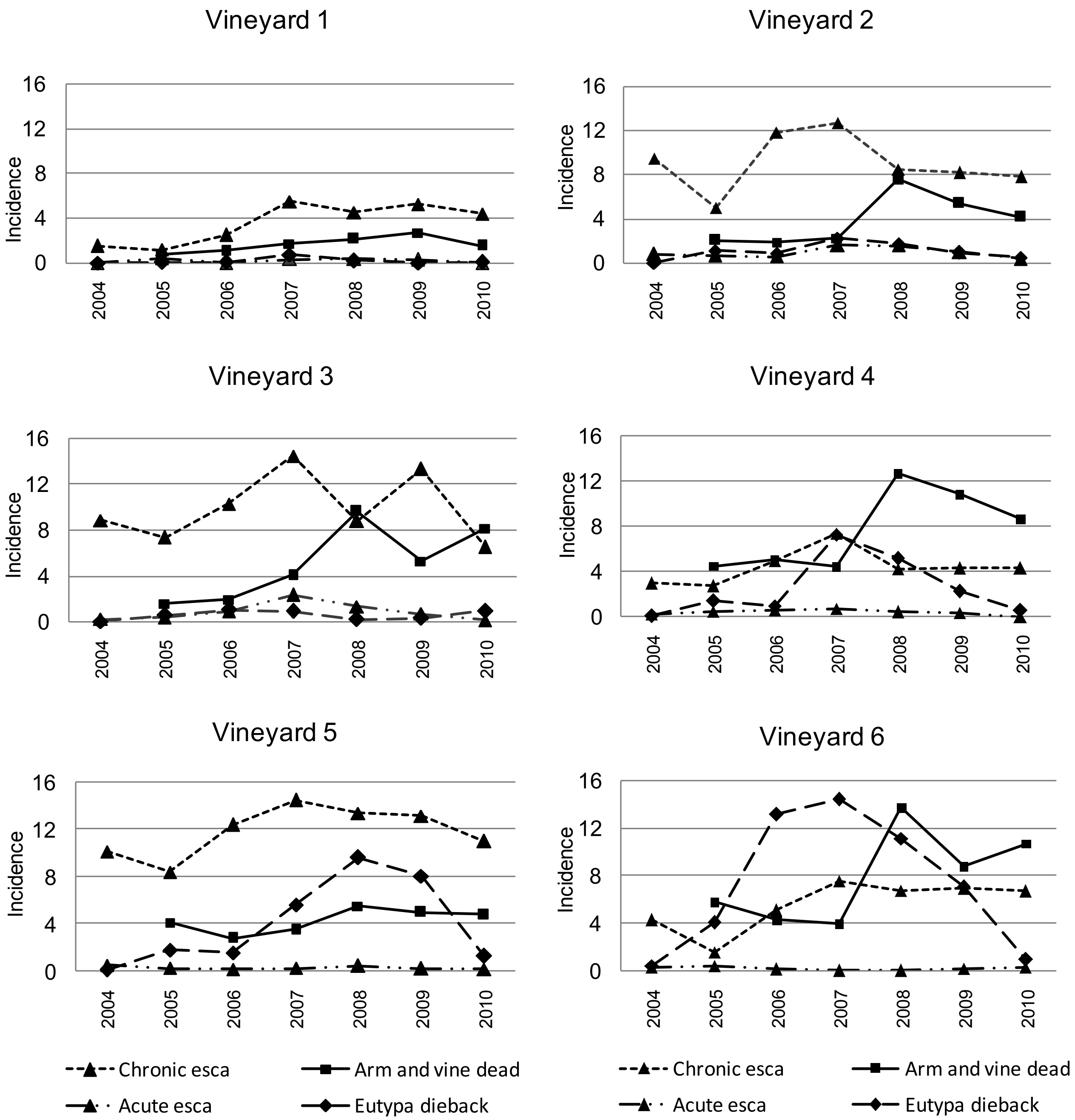

Figure 1. Annual incidence of chronic and acute esca of grapevines, Eutypa dieback foliar symptoms, and dead vines (cordon or trunk) from 2004 to 2010 in the 6 surveyed vineyards.

2009 or 2010 for the six studied vineyards. The results are presented in Table 2.

The value in the Odds Ratio column for the intercept does not indicate an OR, but gives the probability of death for a vine without symptoms in the previous four years, and corresponds to the death incidence shown in Figure 2B. Although the probability of death for vines that had not expressed

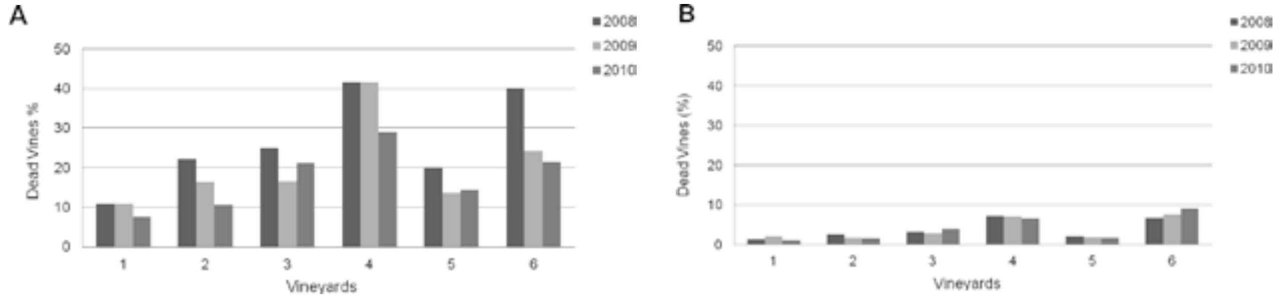

Figure 2. Percentage of dead vines in 2008, 2009 and 2010 among vines either expressing esca symptoms previously (A), or among asymptomatic vines previously (B), in all six vineyards.

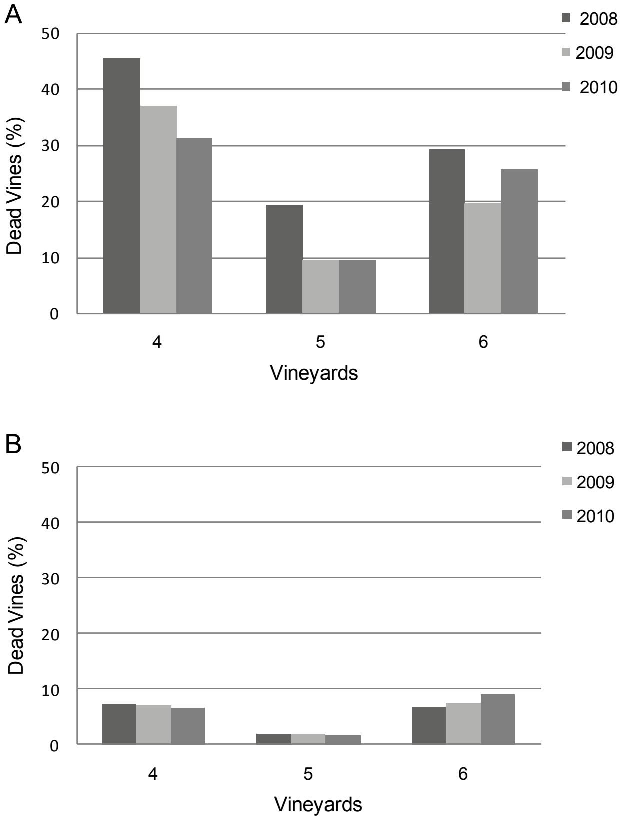

Figure 3. Percentage of dead vines in 2008, 2009 and 2010 among vines either expressing Eutypa dieback foliar symptoms previously (A), or among asymptomatic vines previously (B), in all three vineyards.

symptoms was relatively constant for all three years, most differences were associated with the particular vineyards. The number of selected variables varied between the models from one to four. The presence of symptoms in one of the previous years always increased the probability of death; the year of esca foliar expression preceding vine death was always selected except for the death risk model in 2010 in vineyard 6 and the death risk model in 2009 in vineyard 1 (Table 2). The OR concerned, which varied between 3.3 and 11.3, was generally highest, in comparison with the other selected variables, except for the death risk model in 2008 in vineyard 6.

The variables that were selected for certain models, and which indicated an interaction between two years of foliar expression, had an OR estimate of less than one. This means that the probability of death occurring for vines expressing foliar symptoms for those two years did not show any increase attributable to symptom accumulation. Since, the regression model used esca chronic symptoms (not the acute form) occuring in the years preceding vine death as explanatory variables, and as those results were similar to those presented in Table 2, they have not been presented here.

Eutypa dieback symptom

The results from multiple logistic regressions that were computed with data from the three vineyards showing the highest level of Eutypa dieback incidence, with dead vines as the variable of interest and the symptoms of Eutypa dieback as the explanatory variable are shown in Table 3.

The models are more parsimonious than those used for esca, since only one or two explanatory variables for the previous year of Eutypa dieback foliar expression were selected, except in the case of vineyard 6, for the death year 2008. The OR varied from 2.2 to 11.8. Once again, OR was highest for the variable year preceding death, except in the case of vineyard 5, for vine death in 2010, with the highest OR

Table 2. Odds ratio (Estimate and 95% Confidence Intervals) from the best-fit binary regression logistic model that predicted the risk of vine death in 2008, 2009 or 2010 based on esca symptoms (chronic or acute form) in the previous four years (i.e. S07 = symptoms in 2007) for the six vineyards. *P<0.01, **P<0.001, ***P<0.0001.

Vine-yard	Year of vine death	Variable	Signif-icance	Odds ratio	
				Estimate	95% CI
1	2008	Intercept	***	0.014[a]	*0.008-0.020*
		S06	**	5.802	2.025-16.583
		S07	**	4.658	1.753-11.141
	2009	Intercept	***	0.022	*0.015-0.030*
		S05	***	9.011	3.207-23.299
		S07	***	4.598	2.150-9.166
	2010	Intercept	***	0.010	*0.006-0.016*
		S09	***	11.308	5.263-23.782
2	2008	Intercept	***	0.031	*0.023-0.040*
		S04	***	3.787	2.453-5.819
		S06	***	2.569	1.663-3.947
		S07	***	3.847	2.520-5.866
	2009	Intercept	***	0.018	*0.012-0.025*
		S06	***	4.032	1.944-8.120
		S07	***	5.801	2.916-11.281
		S08	***	9.622	4.802-18.719
		S06×S08	*	0.368	0.137-1.001
		S07×S08	**	0.211	0.080-0.563
	2010	Intercept	***	0.016	*0.011-0.024*
		S06	**	2.459	1.420-4.230
		S08	***	7.700	3.847-15.047
		S09	***	8.523	4.454-16.140
		S08×S09	***	0.101	0.038-0.268
3	2008	Intercept	***	0.035	*0.0260-0.046*
		S04	***	3.784	2.400-5.919
		S05	***	2.769	1.696-4.466
		S06	***	2.184	1.393-3.390
		S07	***	4.234	2.824-6.345
	2009	Intercept	***	0.033	*0.024-0.043*
		S06	*	2.026	1.197-3.596
		S07	***	3.194	1.912-5.284
		S08	***	3.337	1.974-5.582
	2010	Intercept	***	0.043	0.032-0.056
		S07	***	5.819	3.117-10.439
		S09	***	6.165	3.927-9.631
		S07×S09	***	0.237	0.110-0.524
4	2008	Intercept		0.075	*0.060-0.092*
		S04	***	3.532	1.626-7.666
		S05	**	2.591	1.250-5.349
		S06	**	2.355	1.310-4.161
		S07	***	5.985	3.845-9.256
	2009	Intercept	***	0.073	*0.058-0.091*
		S06	***	6.498	3.371-12.427
		S07	***	4.868	2.495-9.190
		S08	***	8.903	4.528-17.144
		S07×S08	**	0.218	0.069-0.701
	2010	Intercept	***	0.072	*0.057-0.090*
		S06	*	2.750	1.203-5.960
		S07	***	2.817	1.506-5.093
		S09	***	4.716	2.597-8.378
5	2008	Intercept	***	0.019	*0.012-0.029*
		S06	***	10.676	3.984-25.807
		S07	***	10.108	5.425-18.939
		S06×S07	*	0.253	0.085-0.811
	2009	Intercept	***	0.021	*0.013-0.032*
		S05	*	2.944	1.174-6.898
		S06	***	2.743	1.301-5.597
		S08	***	5.622	3.011-10.537
	2010	Intercept	***	0.022	*0.013-0.034*
		S08	*	6.170	2.312-14.871
		S09	***	7.010	3.257-14.724
		S08×S09	***	0.224	0.071-0.764

(*Continued*)

Table 2. *Continues.*

Vine-yard	Year of vine death	Variable	Signif-icance	Odds ratio	
				Estimate	95% CI
6	2008	Intercept	***	0.072	0.055-0.092
		S04	***	5.708	2.723-12.028
		S05	*	3.123	1.053-9.125
		S06	***	4.584	2.402-8.654
		S07	***	3.273	1.917-5.473
	2009	Intercept	***	0.079	*0.006-0.101*
		S06	***	2.782	1.067-6.599
		S08	***	4.332	2.416-7.561
	2010	Intercept	***	0.094	*0.073-0.119*
		S06	***	3.780	2.242-6.244

[a] Intercept estimate means probability of death but without previous symptoms.

Table 3. Odds ratio (Estimate and 95% Confidence Intervals) for the best-fit binary regression logistic model that predicted the risk of vine death in 2008, 2009 or 2010 based on Eutypa dieback symptoms in the previous four years (i.e. S07 = symptoms in 2007) for the three vineyards. **P*<0.01, ***P* <0.001, ****P*<0.0001.

Vine-yard	Year of vine death	Variables	Signif-icance	Odds ratio	
				Estimate	95% CI
4	2008	Intercept	***	0.074[a]	*0.059-0.091*
		S07	***	11.417	7.445-17.525
	2009	Intercept	***	*0.073*	*0.057-0.091*
		S07	***	3.822	1.994-7.111
		S08	***	5.604	2.946-10.470
	2010	Intercept	***	*0.072*	*0.056-0.090*
		S07	***	4.547	2.280-8.647
		S09	***	7.708	2.748-21.408
5	2008	Intercept	***	*0.021*	*0.014-0.032*
		S07	***	11.804	5.857-23.346
	2009	Intercept	***	*0.011*	*0.006-0.020*
		S05	*	8.610	1.154-48.307
		S08	***	9.056	3.726-20.190
	2010	Intercept	***	*0.016*	*0.009-0.027*
		S08	***	7.828	2.841-19.952
		S09	**	5.806	1.807-16.054
		S08×S09	*	0.151	0.030-0.788
6	2008	Intercept	***	*0.068*	*0.051-0.088*
		S05	**	2.860	1.348-5.960
		S06	***	4.304	2.238-7.952
		S07	***	5.035	3.003-8.307
		S07×S06	*	0.321	0.136-0.776
	2009	Intercept	***	*0.076*	*0.058-0.097*
		S07	**	2.170	1.271-3.637
		S08	***	3.014	1.791-5.005
	2010	Intercept	***	*0.104*	*0.081-0.131*
		S07	***	2.768	1.727-4.370
		S09	***	3.937	2.395-6.394

[a] Intercept estimate means probability of death without previous symptoms.

being found for the variable S08 (symptoms in 2008). The model also selected the variable indicating interaction between the years of foliar expression, with OR being estimated at less than one.

Necrosis in vines that expressed esca symptoms or Eutypa dieback

The relative area covered by total necrosis was significantly (*P*<0.001) higher in vines expressing Eu-

Table 4. Percentage of the total cross-sectional area of wood corresponding to each type of necrosis, in the cordon alone and in the whole scion of the vine expressing chronic esca foliar symptoms or Eutypa dieback symptoms. Values are means, Minimum and Maximum values of 10 vines with *P* values (Wilcoxon test , **P*<0.01, ***P*<0.001, ****P*<0.0001).

Type of necrosis	Position of the necrosis	No. of vines						*P* value	
		Chronic esca foliar symptom			Eutypa dieback				
		Mean	Min	Max	Mean	Min	Max		
Total necrosis	Cordon	25.7	1.2	71.7	54.8	37.5	73.9	0.0005	***
	Scion	20.5	6.0	38.5	40.4	26.0	64.0	0.0007	***
Sectorial brown necrosis	Cordon	20	0	55.3	51.1	35.5	66	0.0002	***
	Scion	14.7	0	36.1	37.1	26.6	60.3	0.001	**
Altered perimeter	Cordon	34.0	8.2	84.3	60.1	40.0	100	0.006	**
	Scion	25.4	5.5	46.2	42.4	22.9	64.8	0.02	*
White rot	Cordon	3.3	0	46.3	1.5	0	10.4	0.09	
	Scion	4.4	0.04	19.8	1.2	0	7.2	0.04	*

typa dieback foliar symptoms than in vines expressing esca foliar symptoms (Table 4). In both types of diseased vines, cordons showed the highest amount of necrosis and percentage of altered perimeter. In vine cordons expressing Eutypa dieback, over half of the wood tissue was affected by necroses, mainly sectorial brown necrosis. Consequently, the percentage of altered perimeter was greater in vines showing Eutypa dieback symptoms than vines showing esca symptoms. Sectorial brown necrosis was always found in vines expressing Eutypa dieback, but this was not the case for esca vines. Of all vines showing esca symptoms, white rot affected 3.3% and 4.4% of the cross-section surface in cordon and total scion, respectively. The relative area covered by white rot was significantly (P<0.05) lower in Eutypa dieback vines than in esca vines.

Discussion

The logistic models developed to compare the risk of vine death in six vineyards in 2008, 2009 and 2010 revealed great differences in the incidence of esca, Eutypa dieback and dead vines. Within each vineyard, a relative constancy of foliar expression was noticed, despite the annual fluctuation of esca incidence over the seven years. Each vineyard was characterized by its specific sanitary status in response to such multiple factors as wood pathogens, rootstock, climatic and soil factors and vineyard management practices over time (Mugnai *et al.*, 1999, Surico *et al.*, 2006). High rates of esca symptoms occurring in vineyards during the same year could be associated with similar climatic conditions occurring in all the vineyards in the same region. Indeed, a strong positive correlation was found within each vineyard between the sum of rain over the period May-August, and the incidence of vines expressing esca (chronic and acute) (data not shown). These results corroborated those of Marchi *et al.* (2006) who reported that variations in esca-foliar expressions were related to rainfall in spring and summer. As with esca, high rates of foliar symptoms of Eutypa dieback were noticed for the same year and were equally associated with the same climatic conditions.

The high correlation between the incidence of esca in year n, and the percentage of dead vines in year n+1, was consistent with the results of logistic regression. The models showed that the occurrence of foliar symptoms in one of the previous years always increased the probability of vine or cordon death. Stefanini *et al.* (2000) also estimated a greater probability of death for a vine that had expressed esca symptom the year preceding death compared with a vine that had been asymptomatic. They showed that the variable 'sanitary state in the close vicinity" did not improve the conditional probability of the occurence of death, contrary to the occurrence of esca symptom expression. Generally, the models developed in this study also selected one or two earlier years of expression. The results of the logistic model explaining that death depended on the expression of esca symptoms was the same whether the explanatory variables used concerned either the chronic form of esca or the two forms (acute and chronic). This can be attributed to the low incidence of acute esca vines in each of the studied vineyards. Acute esca occurs when particular climatic conditions are met, which was not the case during the present survey period. It should be noted that sometimes OR confidence intervals were not precise, particularly when the corresponding incidence of symptomatic vines was low, since it was not possible to obtain accurate values in this case.

In this study, mortality risk estimated for vines that had expressed foliar symptoms at the field level proved consistent with the results obtained by Péros *et al*. (2008) and Fussler *et al*. (2008).

When Eutypa dieback symptoms were used as the explanatory variables to determine the vine death risk, the number of selected variables in the model was generally lower (one or two) than for esca (two or three). These results suggest that vine death was more rapid when it expressed Eutypa dieback than when it expressed esca. This could be due to the necrosis results since the amount of necrosis and the percentage of altered perimeter in cordon and in total scion were significantly higher in vines showing Eutypa dieback symptoms than in those showing esca foliar symptoms. Two hypotheses can be advanced. First, the higher level of non-functional tissue stops the xylem flow towards the buds during the year after symptom expression. Second, a higher level of greater fungi-toxin production occurs in Eutypa dieback than in esca, with toxins being transported by the sap towards the herbaceous parts of the plant (Andolfi *et al*. 2011). The metabolites produced by *Eutypa lata* also react at the point of production by disrupting the vascular tissue and inhibiting the transport of nutrients (Mahoney *et al*., 2005). Therefore, the toxins can act either at bud burst stage or before, thereby preventing vegetation growth. Whether these toxins are only produced by *E. lata* is still the subject of debate, since Botryosphaeriacaea spp. can also be isolated from the sectorial necroses of vines expressing Eutypa dieback (Luque *et al*., 2009; Lecomte, personal communication).

This study also showed that vine death occurred even if the vines had not expressed foliar symptoms over a period of four to six years. The death of asymptomatic vines could also be related to grapevine cankers caused by different species of Botryosphaeriaceae or Diatrypaceae (Trouillas *et al*., 2010; Úrbez-Torres *et al.,* 2011). One of the surveyed vineyards showed large sectorial necroses, in cross-sectioned cordons, associated with high isolation levels for *E. lata* and Botryosphaeriaceae species (data not shown) without previous foliar symptoms of Eutypa dieback or esca. In addition, individual vineyards showed that when high dead vine incidence occurred among vines that had previously expressed symptoms, it also occurred among asymptomatic vines. These findings suggest that, in both cases, the causes of vine death are similar, but need further elucidation by comparing the wood microflora of previously symptomatic or asymptomatic dead vines. Moreover, these findings could also mean a strong vineyard effect. Each particular vineyard is characterized by its particular environmental (biotic and abiotic) and cultural practices, such as its pruning system which is thought to be a risk factor for esca and *Eutypa lata* (Dumot *et al*., 2004; Surico *et al*., 2006). Studies are currently being undertaken to identify the different factors associated with esca disease and vine death.

The occurrence of white rot in all vine samples having previously expressed foliar symptoms of are indicative of the disease "esca proper". However, white rot was also found in vines that expressed foliar symptoms of Eutypa dieback. These observations show once again the difficulty of characterizing diseases by symptoms when different internal diseases are co-occurring in the same vine (Mugnai *et al*., 1999; Luque *et al*., 2009; White *et al*., 2011).

The comparison of the relative risk of vine death after disease expression between vineyards showed different risks of vine death. Surprisingly, the incidence of vine death was sometimes very low despite the high incidence of esca. This meant that a large number of vines were able to recover after symptom expression, raising the question of the capacity of vine resilience or else the hidden development of the disease, defined by Marchi *et al*. (2006). This capacity to recover after esca symptoms was particularly marked in vineyard 5 which showed a high incidence of esca expression combined with a low mortality. An analysis of the cultural factors should be carried out to highlight the causes associated with that situation. More generally, further studies will be necessary to identify the different factors involved in grapevine trunk diseases explaining differences between vineyards and annual fluctuation of their expression. Other factors, such as vine physiology and climatic conditions, should be taken into account in order to provide an improved understanding and explanation of the risk of vine death.

Acknowledgements

This study was funded by the Burgundy Wine Board (B.I.V.B.) and by the French Ministry of Food Agriculture and Fisheries (CASDAR project). The authors thank all the vine-growers who participated in this study. We also thank Audrey Bastien for her technical assistance and Ray Godfrey for his helpful revision of the manuscript.

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Accepted for publication: April 8, 2013