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RESEARCH PAPER

Overview of grapevine trunk diseases in France in the 2000s

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Summary. The National Grapevine Trunk Disease Survey was conducted in France from 2003 to 2008 to monitor grapevine trunk diseases (GTDs), eutypa dieback and esca/black dead arm (BDA). Data collected from seven regions, 329 vineyards and 12 cultivars were analysed. There were great variations amongst regions in the incidence of GTDs. For esca/BDA, two groups were distinguished: vineyards in Jura and Charentes had greater incidence (93–95%) than those of Bordeaux, Alsace and Bourgogne (54–82%). Incidence increased in Charentes over the 6-year survey, with the highest values being recorded during the last 2 years. For eutypa dieback, all vineyards of Charentes were affected, with 17 to 25% of vines expressing symptoms; for the other regions, 52 to 80% of vineyards were affected, with incidences below 3%. Cultivars Savagnin and Trousseau in Jura were especially affected by esca/BDA. Instead, Ugni Blanc in Charentes was most affected by eutypa dieback. One cultivar could be significantly more affected in one region than in another. The global health status of the vineyards was also investigated. *(i)* For four regions, 82% (Jura) to 87% (Alsace) of the grapevines were healthy, but this percentage decreased steadily (67%) in Charentes. *(ii)* Plants infected by GTDs were 32 and 18% in Jura and Charentes respectively, and only 2.9% in the Bourgogne region. (iii) The unproductive plants, *i.e.* dead, missing, replanted or restored, represented a significant part of the losses (6.6% in Charentes to 9.9% in Jura). The extension of GTDs is discussed with regard to the abiotic and biotic factors that may favour the diseases.

Key words: grapevine cultivar, esca, eutypa dieback, black dead arm, botryosphaeria dieback.

Introduction

Grapevine trunk diseases (GTDs), include three main diseases affecting grapevine wood: eutypa dieback, esca decline and botryosphaeria dieback, which are widespread in the main vine-growing regions of the world (Scheck *et al.*, 1998; Mugnai *et al.*, 1999; Armengol *et al.*, 2001; Rumbos and Rumbou, 2001; Edwards and Pascoe, 2004; Gimenez-Jaime *et al.*, 2006). An increase in the incidence of GTDs over the last 10–15 years has been reported worldwide (Carter, 1991; Chiarappa, 2000; Graniti *et al.*, 2000; Surico *et al.*, 2000; Reisenzein *et al.*, 2000; Úrbez-Torres *et al.*, 2006, 2008, 2009; Sosnowski *et al.*, 2007; Bertsch *et al.*, 2012). In France, until the late nineties, the most common trunk diseases were eutypa and esca, at the

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turn of the century, a third and new disease caused by Botryosphaeriaceous species was identified in Bordeaux (Larignon *et al*., 2001) and in other French vine-growing areas (Panon, 2000). This disease is associated with *Diplodia seriata* ("*Botryosphaeria obtusa*") and *Botryosphaeria dothidea* and was referred to as "black dead arm" (BDA) adopting the same name previously used in Hungary by Lehoczky (1974) for cankers caused on grapevine by other Botryosphaeriaceae species, namely "*Botryosphaeria stevensii*".

In the same years in many vinegrowing countries in the world an increasing incidence of wood cankers caused by various Botryosphaeriaceae species was recorded, referred to as botryosphaeria dieback (Úrbez-Torres, 2011).

Because of the threat that these diseases cause to vineyards, numerous studies were carried out during the last decade to identify the causal fungi and their interactions with the vine in order to develop disease management strategies (Larignon *et al*., 2009; Bertsch *et al*., 2012). In regard to aetiology, the symptoms that occur in the trunk, leaves and berries have been extensively described, revealing that eutypa dieback symptoms differ markedly from those of esca and BDA, with differentiation between the latter two often proving difficult. Lecomte *et al*. (2012) reported that foliar symptoms of esca showed transitory phases that overlapped with some BDA descriptions. For esca, due to the complexity of the symptoms, changes in the definition of this disease have been reported in the literature over the last ten years (Surico *et al*., 2008; Bertsch *et al*., 2012). Two major simplifications in disease terminology were proposed to replace the five initial terms used for esca: brown wood streaking, Petri disease, young esca, esca and esca proper (Mugnai *et al*., 1999). The first simplification was made by Surico (2009), who suggested that the term "young esca" be replaced by "grapevine leaf stripe disease" (GLSD), so that the term "esca" only included white rot (esca) and esca proper. For the three tracheomycotic syndromes, brown wood streaking, Petri disease and GLSD, the term phaeotracheomycotic complex was also proposed, as the same fungi are involved in the three symptomatically different diseases. The second simplification was proposed by Lecomte *et al*. (2012) when they chose not to separate esca symptoms into mild or apoplectic forms, but to use a classification based on a gradual scale of severity, starting from some leaves showing only discolorations up to complete vine wilting.

Eutypa lata, was identified long ago (Carter, 1988) as the main agent of the eutypa dieback, even if, more recently, other species of the diatrypaceae (Trouillas *et al*., 2010; Trouillas and Gubler, 2010) were found to be involved. In the botryosphaeria dieback, according to Úrbez-Torres (2011), 21 different Botryosphaeriaceae species are associated with this disease. Some of them are described to produce in France, beside the typical wood cankers and dieback, also foliar symptoms that resemble closely the grapevine leaf stripe disease, or esca tiger-stripe symptom. On the other hand esca was defined as a complex of diseases, as it involves fungi belonging to various species and families. Generally, the grapevine leaf stripe disease is thought to result mainly from the pathogenic activity of vascular pathogens, e.g. *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, while the wood white decay is mainly caused in Europe by *Fomitiporia mediterranea*. In the present paper the term esca/BDA will be used overall, as a clear distinction between the two tiger stripe symptoms, grapevine leaf stripe disease and BDA, is really difficult in field surveys; hence the authors propose that the GLSD vascular agents and the BDA agents, both producing toxic metabolites, can have a relevant synergistic role in the leaf symptoms formation (Andolfi *et al*., 2011). The involvement of other microorganisms is still a matter of speculation and recent studies indicate that a diverse microflora colonise the wood of esca-diseased grapevines (Bruez *et al*., 2011; Maher *et al*., 2012), but its exact role remains to be determined. It is surely clearly ackwnowledged that the different pathogens can coexist in the same vine (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Bruez, 2013). Finally, the origin of the various terms that define esca should be associated with the microbial species that colonize the wood of grapevines at a specific time. The activity and succession of fungal microflora that occur within the wood may lead to the complexity of the symptoms, thus generating the various terms used in the literature.

The current epidemic spread of esca dates back to the early 1990s (Mugnai *et al*., 1999), according to various European surveys of this disease (Reisenzein *et al*., 2000; Surico *et al*., 2000) while BDA was reported in the early XXI century (Larignon *et al*., 2001; Lecomte *et al*., 2005). Survey results provided vinegrowers and scientists with information on the incidence and evolution of these diseases. The National Grapevine Trunk Diseases Survey was established in

France to monitor the evolution and determine the importance of eutypa dieback and esca/BDA during the period from 2003 to 2008. The survey was conducted in 11 French regions, on 27 varieties of vines and for more than 600 vineyards. A preliminary survey provided information on 256 individual vineyards and, at that time, the mean incidences for eutypa dieback and esca/BDA were 2.23 and 3.25%, respectively (Fussler *et al.*, 2008).

The objective of this study was to assess the status of GTDs in France by comparing disease incidence between regions and cultivars.

Materials and methods

The survey of the French vineyards

The survey of grapevine trunk diseases was done in 329 vineyards randomly chosen across 7 regions of France. In each vineyard, 300 grapevines were assessed in ten randomly chosen groups of 30 vines. Each year during the survey, observations were made on the same grapevines. The grapevines were monitored at two periods of the growing season: in June (at the end of flowering and at the beginning of fruit setting) to assess foliar symptoms of eutypa dieback and in August–September (berry ripening) of esca/BDA. At the same time, dead, missing, replanted, restored and healthy (without GTD symptoms) plants were counted: they represented the unproductive grapevines. The survey was done by employees of FranceAgriMer, the French Plant Protection Agency (French Ministry of Agriculture), Institut National de la Recherche Agronomique (INRA), Institut Français de la Vigne et du Vin (IFV) and vinegrowers associations of each region.

Vineyards were monitored in the regions of Alsace, Bordeaux, Bourgogne, Charentes, Jura, Centre and over Provence-Alpes Côte d'Azur (PACA) over the 6-year period. Some were not monitored one year (Jura in 2003, Alsace in 2006) or two years (Centre in 2007 and 2008). In all, 12 cultivars were included, which generally differed between regions (Table 1).

For each vineyard, the information collected consisted of age, cultivar and rootstocks. Note that, for each cultivar the age value corresponds to the mean of the ages of the vineyards in 2008, in brackets are the minimum and maximum ages of the vineyards (Table 1).

Statistical analyses

The frequency of eutypa dieback and of esca/BDA corresponds to the percentage of affected vineyards. The incidence of eutypa dieback and of esca/BDA represents the percentage of affected vines. For the incidence, the binomial confidence intervals have been calculated by using the package Hmisc of the R software (version 2.14.2) with the command "bioconf" (binomial confidence intervals are calculated because normal estimates are meaningless below 10%).

The plant mortality, i.e. the percentage of dead and missing grapevines, and the replanted and restored plants were also counted. For each result the standard deviation has been estimated.

The non-parametric Kruskal-Wallis test has been used to compare the data obtained with the Cabernet-Sauvignon and Sauvignon cultivars when they were planted in various regions (variances were not equal according to the Levene's test). The pairwise Wilcoxon test has been used after the Kruskal-Wallis test to confirm the results.

Contingency tables allowed us to determine if, for the same cultivar, a relation exists between the rates of esca/BDA and of eutypa dieback. Chi-square test for independence was performed using the package Rcmdr of the R software package (2.14.2).

For eutypa dieback or esca/BDA symptom incidence, three separate bifactorial analyses were performed: a Cultivar × Year of Survey analysis of variance, a Cultivar × Region analysis of variance and a Region × Age analysis of covariance. This design was necessary because most cultivars were peculiar to a region only and the two factors couldn't be jointly analyzed. The percentages of esca/BDA and eutypiose symptomatic expression were transformed into logits to account for the binomial nature of the data.

Results

Comparing frequency and incidence of grapevine trunk diseases between regions

In Charentes, eutypa dieback was recorded in all 27 vineyards surveyed (Figure 1a). For the Alsace, Bordeaux and Bourgogne regions, frequencies varied between 42 and 80% (Figure 1a). In Jura, eutypa dieback was observed in 8% of vineyards in 2004 and 32–38% of vineyards in the years 2005–2007.

Table 1. French vinegrowing regions, cultivars (age, rootstocks) and vineyards used for the survey done by the National Grapevine Trunk Disease Survey (2003–2008).

Vine growing regions	Cultivars	Age of cultivars in 2008	Rootstocks	Number of vineyards monitored for eutypa dieback and esca/BDA	Years					
					2003	2004	2005	2006	2007	2008
Alsace	Pinot Auxerrois	27.9 (16–40)	3309/S04/Teleki8	24	X	X	X		X	X
	Gewurztraminer	25.42 (7–58)	3309/420A/161-49/S04/34EM/Fercal/41B	24	X	X	X		X	X
	Riesling	27.4 (15–55)	3309/S04/161-49	26	X	X	X		X	X
Bordeaux	Cabernet-Sauvignon	24.5 (11–47)	3309/S04/101-14/420A/Riparia	17	X	X	X	X	X	X
	Merlot	24.7 (12–47)	3309/S04/161-14/420A/Riparia	17	X	X	X	X	X	X
	Sauvignon	20.1 (7–31)	3309/S04/101-14/196-17	17	X	X	X	X	X	X
Bourgogne	Chardonnay, 21	41.9 (21–78)	3309/S04/161-14/SBB/5C	28	X	X	X	X	X	X
	Chardonnay, 89	25.8 (16–49)	S04/41B/Teleki	23	X	X	X	X	X	X
	Pinot noir	42.5 (21–78)	3309/S04/161-49/SBB	40	X	X	X	X	X	X
	Sauvignon	26 (20–38)	3309/S04/41B	13	X	X	X	X	X	X
Centre	Sauvignon	23.4 (16–45)	3309/S04/Riparia/SBB	16	X	X	X	X		
Charentes	Ugni blanc	26.8 (10–38)	Paulsen/Rupestris/Fercal/RSB/161-49/41B/R140	27	X	X	X	X	X	X
Jura	Poulsard	26.7 (15–38)	3309/S04/101-14	25		X	X	X	X	X
	Trousseau	22.5 (15–43)	3309/S04/101-14	25		X	X	X	X	X
	Savagnin	23.9 (14–51)	3309/S04	23		X	X	X	X	X
Provence-Alpes Côte d'Azur	Cabernet-Sauvignon	24.1 (15–29)	S04/R110/Paulsen	10	X	X	X	X	X	X

The incidences of eutypa dieback in the vineyards of Charentes varied from 17 to 26% between years (Figure 2a). From 2003 to 2008, there was a tendency for the incidence to decrease. The three highest percentages (above 21%) were recorded in 2003–2005 and the three lowest (below 20%) in 2006–2008. For the Alsace and Bordeaux vineyards, the incidence of eutypa dieback was always below 4%, the lowest value was obtained the first year of the survey (2003) and the two highest in 2007 or 2008 (Figure 2a). In the Bourgogne vineyards, the incidence was less than 1% in 2004, 2006 and 2007; the maximum was 1.95%

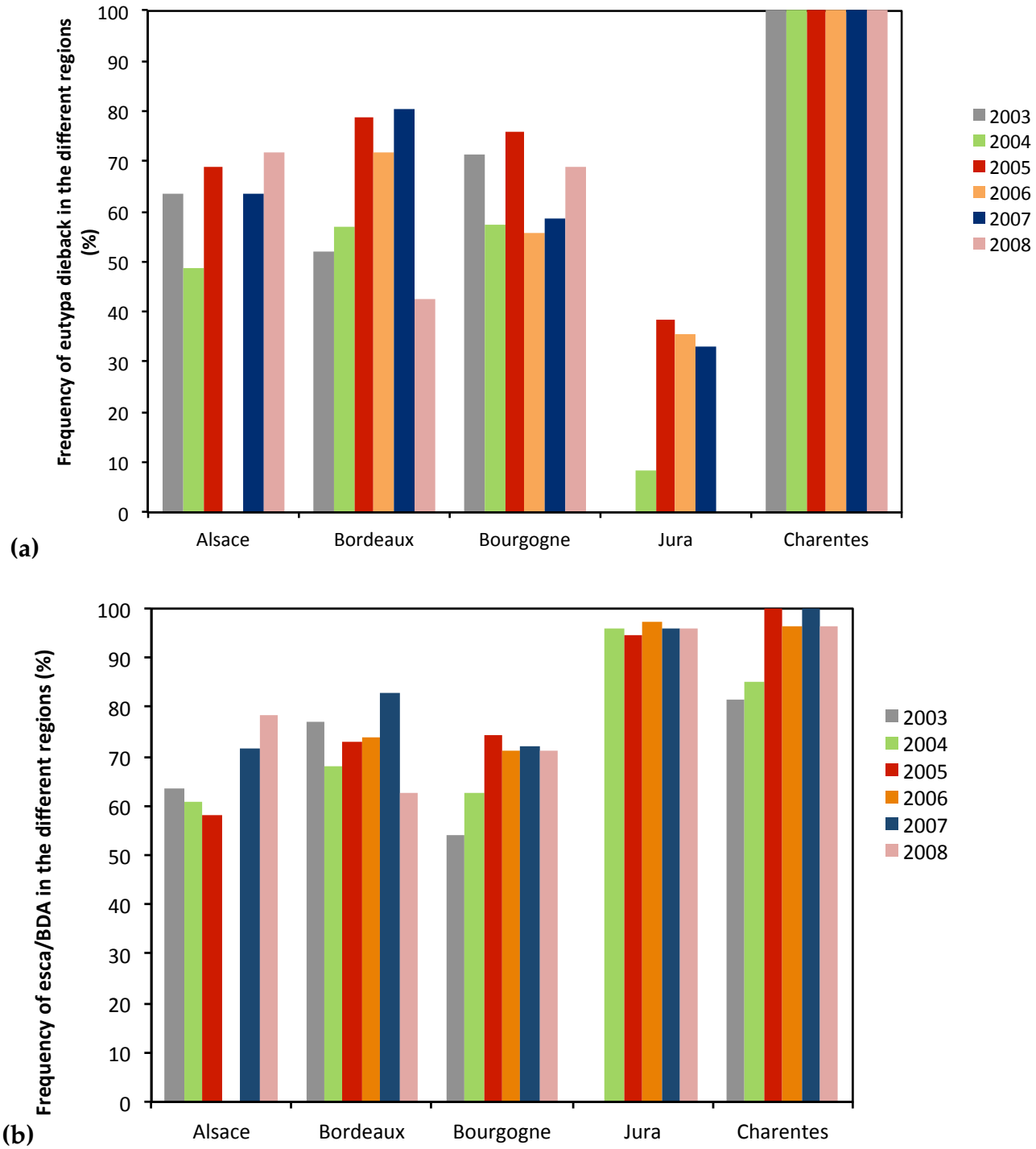

Figure 1. Frequency of eutypa dieback (a) and of esca/BDA (b) in the five regions, Alsace, Bordeaux, Bourgogne, Jura and Charentes surveyed over the six-year period.

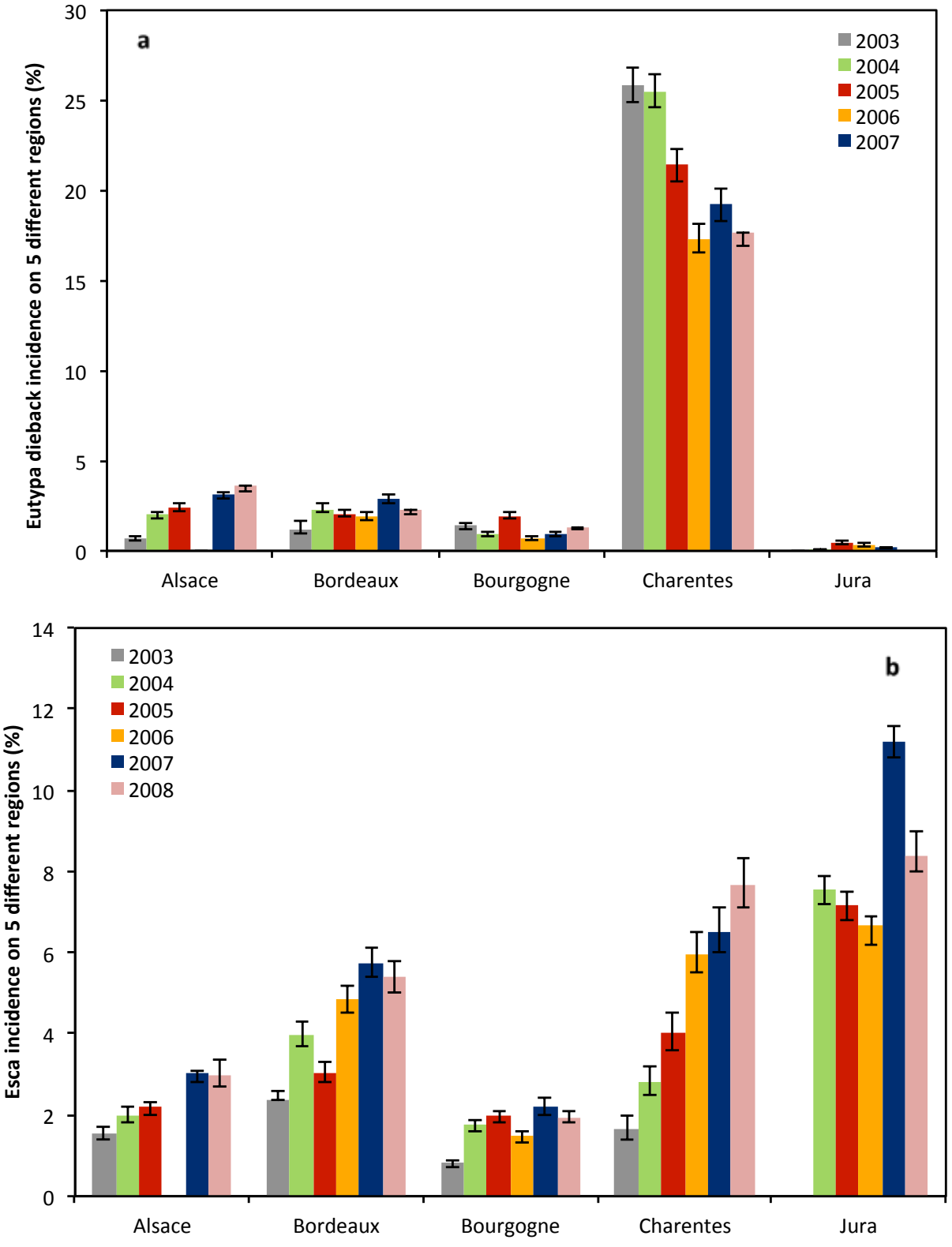

Figure. 2. Eutypa dieback (a) and esca/BDA (b) incidences in the five regions: Alsace, Bordeaux, Bourgogne, Jura and Charentes surveyed over the six-year period. Confidence intervals are indicated on the bars.

in 2005. For the Jura region, the incidence of eutypa dieback in the vineyards was always below 0.5%.

The Jura and Charentes regions had higher frequency and incidence of esca/BDA compared with that of the Alsace, Bordeaux and Bourgogne regions (Figures 1b and 2b). In the Jura vineyards, the frequency was greater than 95% and the incidence varied from 7 to 11%. In Charentes, the frequency of vineyards with esca/BDA was around 81–85% the first two years of the survey, with all vineyards affected in 2005 and in the following years frequency varied from 96 to 100%. The incidence of esca/BDA in vineyards increased with years in Charentes from 2 to 8%.

For the three other regions, the mean frequencies of esca/BDA were relatively similar; 66% in Alsace, 73% in Bordeaux and 68% in Bourgogne. For all five regions, the lowest values of incidence were obtained in 2003 and the highest in 2007 or 2008.

Comparing incidence of grapevine trunk diseases between grapevine cultivars

In the Charentes region, the incidence of eutypa dieback in cv. Ugni Blanc vines (22%) was significantly higher than the other cultivars in other regions in the survey; Cabernet-Sauvignon and Sauvignon (2.5–3%) and all others were below 1% (Figure 3a).

The greatest incidence of Esca/BDA was recorded in cv. Savagnin and Trousseau (10%) in the Jura region (Figure 3b). In contrast, less than 2% of vines of cv. Pinot and Chardonnay in Bourgogne and in cv. Merlot in Bordeaux showed disease. Four percent of Chardonnay vines, planted in the department of the Yonne ("Chardonnay 89" on Figures 3a and 3b) in Bourgogne were recorded with esca/BDA symptoms, as compared with 1% of cv. Chardonnay vines, which were planted in the department of the Côte d'Or ("Chardonnay 21" on Figures 3a and 3b) in the same region. Except for the cv. Sauvignon, from the Bordeaux vineyards, that had 7% of vines attacked, for the other cultivars from the other regions, 3 to 6% of vines expressed esca/BDA foliar symptoms.

The incidence of esca/BDA was significantly greater on Cabernet-Sauvignon grapevines in Provence-Alpes Côte d'Azur than in Bordeaux in 2003–2006 (Kruskal-Wallis test, $P<0.001$) but not in 2007 ($P=0.191$) and 2008 ($P=0.597$) (Figure 4).

When the esca/BDA incidences on Sauvignon cultivar planted in the Bordeaux, Bourgogne and Centre regions are compared over the 6-year survey, no significant differences were observed (Figure 5).

Assessment of grapevine losses from 2003 to 2008

The percentage of healthy plants in Charentes was 67% compared with 89% for Bourgogne, 87% in

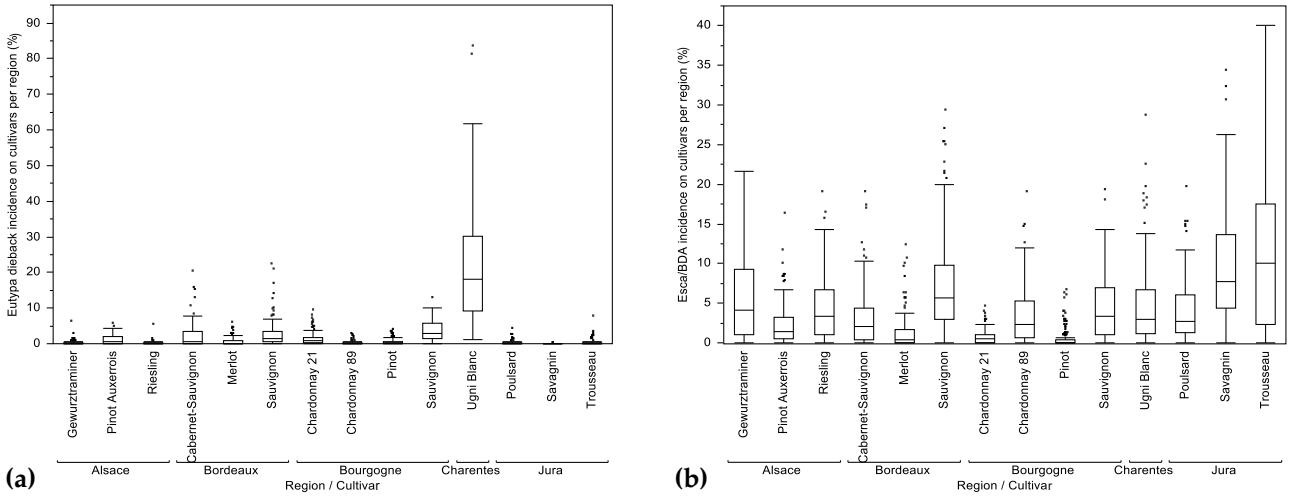

Figure 3. Eutypa dieback (a) and esca/BDA (b) incidences of different cultivars planted in the five regions: Alsace, Bordeaux, Bourgogne, Charentes and Jura surveyed over the six-year period. Chardonnay 21 and 89 are planted in the Côte d'Or and Yonne departments of France respectively. Data are expressed as a box-and-whisker plot showing median, interquartile range (IQR) and extreme values.

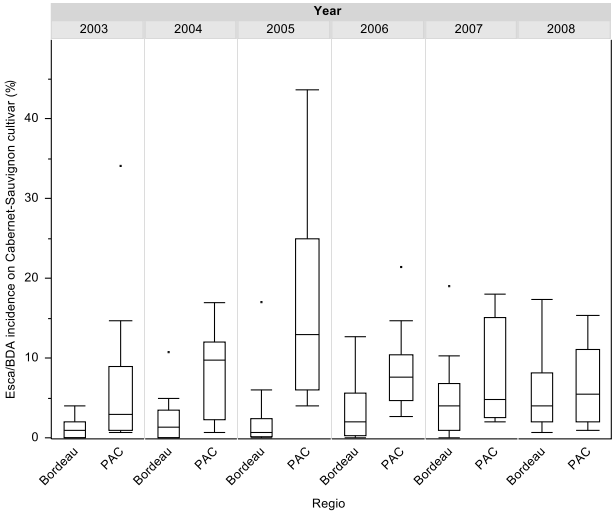

Figure 4. Esca/BDA incidence on Cabernet-Sauvignon cultivar planted in the Bordeaux and Provence-Alpes Côte d'Azur regions (PACA) regions. Data are expressed as a box-and-whisker plot showing median, inter-quartile range (IQR) and extreme values.

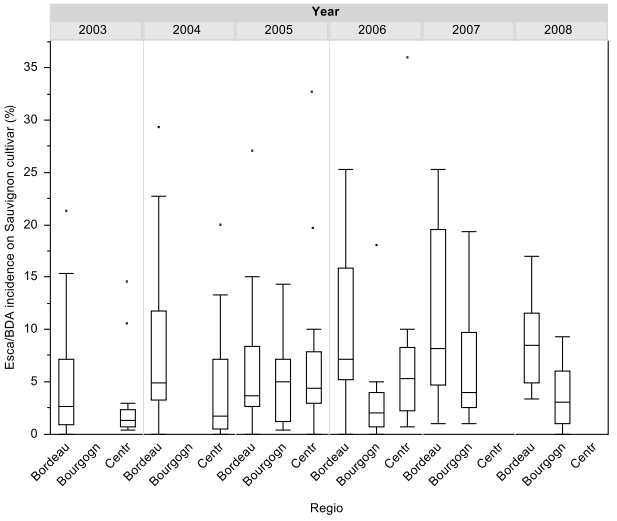

Figure 5. Esca/BDA incidence on Sauvignon cultivar planted in the Bordeaux, Bourgogne and Centre regions. Data are expressed as a box-and-whisker plot showing median, inter-quartile range (IQR) and extreme values.

Alsace, 86% in Bordeaux and 82% in Jura. In Alsace, dead and missing vines accounted for 0.6 and 1.2% of vines, respectively. For the other regions, the incidence of dead plants was relatively similar, from 1.1% in Bourgogne to 1.7% in Charentes. The same trend was observed for the missing vines, the values varied from 2.5% in Bourgogne to 3% in Jura. Replanted vines were most numerous in Jura (5%), Alsace (4.9%) and Bourgogne (4.6%), the lowest percentage was in Charentes with 1.2%. The highest incidence of restored plants was in Alsace (1.4%) and Charentes (0.9%) with only 0.05% of vines restored in Bourgogne (Table 2a).

Regarding the two GTDs surveyed over the six-year period, 21% of the vines expressed symptoms of eutypa dieback in Charentes, in the 4 other regions that value was reduced to 1–3% in Bourgogne, Bordeaux and Alsace and was only 0.3% in Jura. For esca/BDA, 8% of vines expressed leaf symptoms in Jura, that percentage was reduced by half in Charentes and Bordeaux (4–5%), with the lowest value obtained in Bourgogne (1.7%).

Table 2b shows the percentages of unproductive plants, they were relatively similar for all five regions since they ranged from 7% in Charentes to 10% in Jura. For vines affected by GTDs, the highest values were in Charentes (26%) and Jura (9%), while only 3% of the plants were diseased in Bourgogne.

The total percentage of unproductive plants and GTDs affected grapevines across all regions ranged from 33% in Charentes to 11% in Bourgogne (Table 2b).

Link between the diseases and various parameters

For each cultivar, the relations between esca/BDA and eutypa dieback incidences are shown in Table 3. Among the 12 cultivars, a significant relation between the two diseases was only observed for the cultivars Trousseau (Jura region: $0.05>P>0.01$) and Sauvignon (Bordeaux region: $0.05>P>0.01$).

The analyses of variance and covariance (Table 4) showed that for esca/BDA and eutypa dieback expression of the symptoms, there are differences between the regions, between the year and between the cultivars. The age of the vineyards is not a variable associated with the percentage of symptomatic expression of eutypa dieback and esca/BDA ($P>0.05$). The effect of the cultivars is the strongest one ($P<10^{-15}$), followed by that of the region ($P<10^{-9}$) for both diseases. The effect of the year of survey was very significant for eutypa dieback ($P=0.0016$) but only marginally for esca/BDA ($P=0.036$).

Table 2a. Percentage of grapevines that were: healthy, affected by grapevine trunk diseases (eutypa dieback or esca/BDA), dead, missing, replanted, restored in five French regions (surveyed from 2003 to 2008).

Region	Healthy plants	Dead plants	Missing plants	Replanted	Restored plants	Eutypa dieback affected plants	Esca/BDA affected plants
Alsace	87.2 ± 3	0.6 ± 0.4	1.2 ± 0.8	4.9 ± 1.4	1.4 ± 1.8	2.3 ± 0,3	2.3 ± 1.6
Bordeaux	85.7 ± 5.6	1.2 ± 0.9	3.0 ± 2.4	2.9 ± 1.4	0.8 ± 2.1	2.1 ± 1.2	4.2 ± 1.7
Bourgogne	88.8 ± 5	1.1 ± 0.6	2.5 ± 2.8	4.6 ± 8.4	0.1 ± 0.1	1.2 ± 0.6	1.7 ± 0.9
Charentes	67.4 ± 9.3	1.7 ± 0.5	2.8 ± 0.5	1.2 ± 0.9	0.9 ± 1.8	21.2 ± 5.9	4.8 ± 1.8
Jura	82.2 ± 5.5	1.0 ± 0.9	3.0 ± 1.1	5.0 ± 1.8	0.2 ± 0.4	0.3 ± 0,2	8.2 ± 3.0

Table 2b. Percentage of grapevines that were unproductive (dead, missing, replanted, restored are together) or affected by grapevine trunk diseases (eutypa dieback and esca/BDA) in five French regions (surveyed from 2003 to 2008).

Region	Unproductive plants	GTDs affected plants	Total
Alsace	8.1	4.6	12.8
Bordeaux	7.9	6.3	14.3
Bourgogne	8.4	2.9	11.2
Charentes	6.6	26.0	32.6
Jura	9.9	8.5	18.4

When the two variables, region and cultivar, are studied jointly to compare the rate of attack, the results show that the esca attack rate differs depending on the two variables. For eutypiose, the attack rate differs only depending on the variety.

The LSD test results confirmed the differences obtained by the ANCOVA and ANOVA tests.

Discussion

These results show that the GTDs, eutypa dieback and esca/BDA, are present in all French vineyard regions in the survey. However, depending on the region, there were variations in the frequency and incidence of GTDs in the vineyards. Esca/BDA frequency was greater in Jura and Charentes, than in Bordeaux, Alsace and Bourgogne. Generally, from one year to another, the incidence did not fluctuate, except in the Charentes region where a constant increase occurred over the 6 years. For all regions, the highest frequencies were recorded during the last two years, 2007 and 2008. Does that mark the beginning of an epidemic that will develop slowly but steadily over the years? Data obtained in 2009 to 2012 from another survey performed in some French regions (Grosman and Doublet, 2012), i.e. increase of esca/BDA incidence, suggest that this hypothesis is possible. However, the increase observed in the present study seems to be lower than that reported by Reisenzein *et al.* (2000) in Austria. They indicated that the number of vines with external symptoms increased by an average annual rate of 2.7% in 6 years, but in certain vineyards the increase was between 2% and 20%.

Charentes was the only region with significant levels of eutypa dieback, with all its vineyards having from 17 to 25% incidence of symptoms for all 6 years. In the Alsace, Bordeaux and Bourgogne regions, the frequencies fluctuated over the years (from 52 to 80%) but were always lower than in Charentes. However this could also be due to a particular susceptibility of cv. Ugni Blanc, unique to this region. Indeed, our results show that the effect of the cultivar is stronger than that of the region. Unfortunately, most of the cultivars are peculiar to only one of the regions surveyed and the relative influence of the two could only be measured for Cabernet-Sauvignon and Sauvignon Blanc. The incidences were relatively low, as generally less than 3% of the grapevines expressed symptoms. Jura seems relatively free of this disease

Table 3. Correlation between esca/BDA and eutypa dieback using a contingency table chi-squared test.

Region	Cultivar	Data Chi-2 test	Data table
Alsace	Auxerrois	1.79	3.84
	Riesling	0.08	3.84
	Gewurztraminer	0.21	3.84
Bordeaux	Sauvignon	4.17	3.84[a]
	Merlot	1.8	3.84
	Cabernet-Sauvignon	0.003	3.84
Bourgogne	Chardonnay 21	0.7	3.84
	Chardonnay 89	0.002	3.84
	Pinot	0.65	3.84
	Sauvignon	0.04	3.84
Charentes	Ugni blanc	0.001	3.84
Jura	Poulsard	3.3	3.84
	Savagnin	0.13	3.84
	Trousseau	5.66	3.84 [a]

[a] Link between esca /BDA and eutypa dieback.

since the frequency was particularly low (8 to 38%), and no more than 0.46% of grapevines expressed symptoms of eutypa dieback. So these data suggest that, unlike esca, eutypa dieback was not increasing in France. To support that observation, even in the most affected region, Charentes, the lowest values of grapevines expressing eutypa dieback were only obtained over the last three years of the survey (2006 to 2008). However, as symptoms of eutypa dieback may fluctuate considerably, we suggest that the disease needs to be surveyed over a longer period.

To obtain an overview of the situation of GTDs in France, it should be borne in mind that, from one year to another, the same grapevines do not necessarily express foliar symptoms. So, when a period of several years is considered, the number of "foliar symptomatic vines at least once" is far more significant. For instance, Surico *et al.* (2000) indicated that, in a 6-year survey in Italy, the annual incidence of esca affected vines ranged from 11 to 19%. But they found numerous different sequences of symptom expression of vines over that period, with a cumu-

Table 4. Correlations for eutypa dieback or esca/BDA symptoms between regions and age of grapevines, cultivars and year and between the cultivar and the region were calculated by analysis of covariance (ANCOVA). The values with an asterisk are significantly different at $\alpha = 0.05$.

P-values for the esca/BDA attack data	
	ANCOVA
Region	1.63E-10 *
Age	0.7119

P-values for the eutypa dieback attack data	
	ANCOVA
Region	4.36E-10 *
Age	0.9847

P-values for the esca/BDA attack data	
	ANCOVA
Cultivars	2.00E-16 *
Year	0.036 *

P-values for the eutypa dieback attack data	
	ANCOVA
Cultivar	2.00E-16 *
Year	0.0016 *

P-values for the esca/BDA attack data	
	ANOVA
Cultivars	2.00E-16 *
Region	0,001 *

P-values for the eutypa dieback attack data	
	ANOVA
Cultivar	2.00E-16 *
Region	0,331

lative incidence of around 50%. In another Italian survey, Surico *et al.* (2006) again reported an increase in esca over time in three vineyards in Siena (50% over 11 years), Ravenna (30% over 6 years) and Florence (51% over 5 years). In France, Grosman and Doublet (2012) reported that in a 10-year survey in Bourgogne in a vineyard with low incidence (3–4% each year), a total of 21% of vines expressed esca/BDA at least once over that period. The expression of foliar symptoms is not neutral, as shown by Guérin-Dubrana *et al.* (2013, this Journal issue) showed that mortality due to esca is consistently associated with the foliar symptom expression the year before grapevine death. For eutypa dieback, symptoms are also a risk factor for death, greater or equal to that of esca. Only two cultivars out of twelve showed a link between eutypa dieback and esca/BDA. So, it does not seem that the two diseases were prominent at the same time within a vineyard.

The present study showed that the trends in the symptomatic expression of GTDs were relatively similar within each of the five geographic regions, but frequencies and incidences differed greatly between regions. We identified several factors that may be responsible for such differences: the cultivars, regions and year of survey. Our study clearly shows that some of the cultivars were affected more than others by esca/BDA, i.e. Savagnin, Trousseau, or eutypa dieback, i.e. Ugni Blanc. Although this factor cannot be separated from the region, its influence was strong. We observed that Cabernet-Sauvignon can be significantly more affected in a certain French region, i.e. Aquitaine, than in another, i.e. Provence-Alpes Côte d'Azur. However, for the cultivar Sauvignon, we did not observe differences between the Bordeaux, Centre or the Bourgogne regions.

For eutypa dieback, Sosnowski *et al.* (2007) reported that differences also occurred. They reported that foliar symptoms caused by *E. lata* on the cultivar Shiraz varied from year to year in South Australia in a 6-year survey and that, although trends were similar for vineyards within geographical regions, differences were observed between the two regions in which the experiments were conducted. All these results indicate that besides "varietal susceptibility", other factors, such as climate and soil, may be involved.

For other countries, White *et al.* (2011) reported that esca was found on a range of grapevine cultivars planted in South Africa, with some of them being the same as those surveyed in France, *i.e.* Chardonnay, Cabernet-Sauvignon, Merlot. However, as the extent of the disease was not mentioned, it was not possible to compare them. A study of the susceptibility of one cultivar planted in different countries, for instance, Cabernet-Sauvignon in France, Italy and the USA, Riesling in France and Austria (Reisenzein *et al.*, 2000), would certainly be useful in characterising the factors that are the more representative of each area. It would subsequently help to determine the main factors that favour GTDs.

We found that regions and the year of survey have an indirect effect on the disease. Each region has its own different climate: Alsace, for instance, has a continental climate and Bordeaux an oceanic one. Although we have not investigated this point, climate certainly has an influence on the development of GTDs. Surico *et al.* (2006) suggested that climate changes have exacerbated the esca problem. The intensity and variations of rainfalls over the years have a strong influence on the disease. Marchi *et al*. (2006) reported that, throughout a growing season, the number of grapevines that remained asymptomatic was inversely related to the rainfall in May–June or in summer. Chronic esca expression is associated with hot periods in summer following rainfalls whereas, during hot, dry summer periods, severe esca (apoplexy) was more common (Surico *et al*., 2000).

Another important point is temperature, which may differ from one year to another. Lecomte *et al.* (2012) observed that, whatever the vineyard or year of survey in the Bordeaux region, the appearance of esca-leaf symptoms increased regularly from the beginning of June until the end of July. Afterwards, the rate of leaf symptom occurrence decreased, with certain symptoms remaining visible until September. Such an evolution of leaf symptoms could be associated with the progressive increase of mean temperatures in early summer.

As regards eutypa dieback, it is well known that the susceptibility of grapevine wounds to infection by *E. lata* spores is favoured by cool winter conditions (Munkvold and Marois, 1995; Chapuis *et al.*, 1998). Sosnowski *et al.* (2007) carried out a 6-year study in South Australia, to study the influence of climate on foliar symptoms of eutypa dieback on grapevines, cv. Shiraz. They found that various parameters, such as winter rainfall 18 months earlier, increased temperatures in spring, and very high and very low rainfalls in October may have had an influence on the disease. These results led the authors

to set up a model predicting the incidence of foliar symptoms of eutypa dieback.

One of the main results from this study is the status of vines growing in the five French regions we surveyed. It was found that 82% (Jura) to 87% (Alsace) of vines were healthy, but only 67% of vines were healthy in Charentes. These relatively low values of healthy plants should be regarded as the consequence of two kinds of aspects. Firstly plants were affected by GTDs in 18 and 32% in Jura and Charentes, respectively, and only 3% in Bourgogne. Secondly, unproductive plants, i.e. dead, missing, replanted or restored grapevines, represented a significant part of losses in Charentes (6.6%) and Jura (9.9%). In addition to the yield losses, decrease in wine quality (Dubos and Larignon, 1987; Lorrain *et al.*, 2012) and death of vines caused by GTDs, another part of the losses is due to these diseases, because vinegrowers currently uproot affected plants, replanting or restoring them whenever possible. When grapevines are replanted, there is a waiting period of at least three years before the first harvest. Although the costs associated with trunk diseases are often difficult to quantify, in South Australia, eutypa dieback has been estimated to cause yield losses of at least 860 and 740 kg ha^{-1} for the Shiraz and Cabernet-Sauvignon varieties (Wicks and Davies, 1999). For Shiraz alone, production losses in Australia were equivalent to 20 million Australian dollars. In California, economic losses of up to US$260 million per annum have been attributed to the same disease (Siebert, 2001). In France, relevant assessments have not been made, but it was estimated that GTDs induced losses of around 6–7 million euros per year in the Loir-et-Cher alone (P. Martin-Lalande, personal communication). Hofstetter *et al.* (2012) estimated that the worldwide annual financial cost of the replacement of death plants due to GTDs is in excess of 1.132 billion euros. It is thus clear that these diseases are a major threat for the wine-producing regions everywhere.

To conclude, the present work indicates that GTDs are of major concern for the sustainability of certain French vineyards and vine cultivars. We have mentioned some factors that seem to be of major importance in the development of these diseases. Further studies have to be undertaken to determine accurately their impact on the development of GTDs; presumably, also other factors are involved (Lecomte *et al.*, 2011). Surico *et al.* (2004) identified the use of poor quality planting material and the changes in the use of fungicides as some of the possible causes for the upsurge of esca in Tuscany, Italy. According to Peros *et al.* (2008) the use of good quality planting material and the choice of agronomic practices that favour grapevine longevity appear to be useful strategies to reduce the incidence of esca. The challenge is big one, at the same time, GTDs have created a great apprehension in viticulture, as shown by recent demonstrations in France (M. Bessard, personal communication). One French deputy recently spoke of the "new phylloxera" of the 21st century in his declaration on GTDs at the National Assembly and vinegrowers called for rapid control solutions. However, because grapevine trunk diseases involve a range of biotic and abiotic factors, finding solutions would require managing most of the above-mentioned factors. A systemic approach would seemingly be needed to resolve this complex issue.

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