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RESEARCH PAPERS - 10TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Esca of grapevine and training practices in France: results of a 10-year survey

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Summary. Esca is a widespread and damaging grapevine trunk disease in France. A survey was undertaken to identify relevant cultural factors that may influence symptom severity. Preliminary observations in the Aquitaine region confirmed the complex interactions among cultivar, vine training system, and climate, so the study was expanded to the national level to help account for esca in different wine growing regions. Twenty-five vineyard plots were examined. The plots were comparable by pairs, with the same cultivar (or cultivar with similar levels of susceptibility in a few cases), with the same age and similar soil and climatic environments, but with different training or pruning systems. Esca was the predominant trunk disease and prevalence was assessed by visible symptoms on leaves and on wood. Training systems with long arms (or cordons) were generally less affected by the disease than those with short or no arms. Pruning also played a major role, with a trend of less severe symptoms associated with less pruning. The study confirmed that foliar symptoms reveal the presence of the disease, but cannot be considered a reliable indicator of the disease impact in all situations. This study also confirmed: i) that vine training and pruning options may greatly influence the severity of esca, ii) that increasing the length of cordons may minimize the consequences of the wood necroses, and, iii) that simplifications of the woody vine structure (resulting from adoption of modern training and pruning options) may have favoured the development of esca.

Keywords: damage, grapevine trunk diseases, pruning, trellising, wounds.

Introduction

Over the last two decades, esca damage of grapevines has dramatically increased in France (Lecomte *et al*., 2012b; Bruez *et al*., 2013). This trunk disease affects grapevine wood, causing partial or total death of vines. It is commonly associated with a complex of fungal pathogens, ascomycetes and basidiomycetes, among which *Phaeomoniella chlamydospora, Phaeoacremonium minimum* and *Fomitiporia mediterranea* are often reported (Larignon *et al*., 1997; Mugnai *et al*.,1999). Studies have also demonstrated that species in the Botryosphaeriaceae, causing Botryosphaeria dieback, can also be involved in the process of wood degradation of esca-affected vines (Mugnai *et al*., 1999; Berraf and Péros, 2005; Aroca *et al*., 2006; Calzarano and Di Marco, 2007; Péros *et al*., 2008; Luque *et al*., 2009; Maher *et al*., 2012; Ammad *et al*., 2014; Choueiri *et al*., 2014). Using modern molecular techniques, many trunk pathogens, wood-colonizing fungi, and wood-colonizing bacteria have been identified within apparently healthy grapevine wood (Bruez *et al*., 2014; Bruez *et al*., 2015). However, the roles of many of these microorganisms remains unknown. Examining the internal wood symptoms, in cross or longitudinal wood sections of vines with trunk diseases, often characterizes adult vines as having diverse shapes and colours of wood discolourations, possibly due to mixed infec-

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tions by distinct fungi causing inner wood necroses (Larignon *et al.*, 1997; Choueiri *et al.*, 2014).

Esca is characterized by diagnostic foliar symptoms, which are distinct from those of other trunk diseases (Viala, 1926; Arnaud and Arnaud, 1931; Surico *et al.*, 2006). Based on the views of many authors, esca is described as having two forms. The 'mild form' is characterized by discolourations and/or scorching (drying zones) on the margins and/or in between the veins of leaves, producing an overall striped appearance on at least part of affected vines. The 'apoplectic form' is characterized by a sudden and severe collapse of entire vines (Mugnai *et al.*, 1999; Lecomte *et al.*, 2012a). Other authors have described esca as a complex of wood diseases, some of which also show foliar symptoms (Mugnai *et al.*, 1999; Surico *et al.*, 2009). However, recent observations propose a different interpretation, showing that esca symptoms could not be characterized as only mild or apoplectic forms (*sensu latu* Viala, 1926; Arnaud and Arnaud, 1931), but might instead be more accurately classified as a gradation of severity, ranging from some symptomatic leaves to total vine collapse (Lecomte *et al.*, 2012a). These observations also indicated the importance of a third type of symptom, present in the external vine wood and often neglected in the past. This symptom, first described by Arnaud and Arnaud (1931), appears as longitudinal and superficial discolourations of the young wood vessels located just below the bark, which are easily visible in the growing season by peeling off the bark. Also called 'wood stripe', this symptom may result from fungal invasion leading to vessel occlusions and/or to sudden disruption of the sap flow occurring with water stress (due to the infection) during a period of water restriction in late spring or summer (Lecomte *et al.*, 2012a).

According to the results of a national survey in France in the beginning of this century (Grosman and Doublet, 2015), an average of approx. 13% of vines in 2015 were unproductive due to the two main grapevine trunk diseases (GTDs), esca and Eutypa dieback. The impact of these diseases was only about 3% in 2003 (Grosman and Doublet, 2012), which indicates an increase. The disease of greatest concern was esca. In this survey, national incidence of GTDs varied greatly between vineyards. For example, in the Bordeaux area in 2008, percentages of affected vines among 18 plots of the same age from varied from 2 to 41%, and for two Cabernet Sauvignon vineyards planted in 1982 from 3 to 41%. These large differences among vineyards were also observed in 2007 in the Bordeaux area, in another survey (Lecomte *et al.*, 2008).

These local observations, showed that factors such as varietal susceptibility, vine vigour, fertilizer applications (Calzarano *et al.*, 2014; Calzarano and Di Marco, 2018), topography and cultural practices, alone or combined, may contribute to the large variations of disease incidence. All of these observations contributed to a study of causal factors, which began in the Bordeaux area in 2007. The aim was to better understand the roles of abiotic factors in the development of grapevine trunk diseases, particularly focusing on esca.

When a disease causes severe damage on a crop in a given situation, one step to understanding the inciting factors consists of comparing the agronomic context of comparable fields in close vicinity. In the case of esca, the goal is to identify predisposing or aggravating factors (Péros, 1995), as many biotic or abiotic factors may influence the development of the disease and the variability of its damage (Surico *et al.*, 2004; Lecomte *et al.*, 2012b; 2018b). The age of the vines and the susceptibility of some cultivars are two well-known biotic factors (Mugnai *et al.*, 1999). According to Surico *et al.* (2000), expression of esca is also favoured by heavy and wet soils. Soils with high water content and nitrogen availability were reported to be more favourable to the development of esca in grapevine in the Aquitaine region (Destrac *et al.*, 2007). Robotic and Bosantic (2007) reported in Serbia an influence of vineyard topography, showing a greater esca incidence in the low and flat areas of a vineyard partly planted on a slope. However, these agronomic factors, which are already known, may not account for all the observed variability between adjoining vineyards. Other factors, including cultural practices, such as vine training and pruning, may also play significant roles in the variability of esca in vineyards (Lecomte *et al.*, 2008, 2011, 2012b). These cultural practices are the subject of the study reported in the present paper.

Pathogens are the often the main factor involved in the development of disease symptoms. However, the presence of pathogens in grapevine wood does not necessarily lead to rapid decline. With age, inner wood necrosis is generally present in most vines, whether or not they are leaf-symptomatic (Dumot, 2007; Lecomte *et al.*, 2008; Hortsfetter *et al.*, 2012; Bruez *et al.*, 2014). Infections can be very common and inoculum availability seems not limiting, at least in some regions. However, for esca, the relative proportion of inner necrosis from which pathogens can be isolated is frequently a key factor explaining the decline and severe symptoms (Maher *et al.*, 2012), even if it is not always related to the expression of foliar symptoms or decline. Using image analysis of trunk longitudinal sections, Lecomte *et al.* (2008) showed that leaf-symptomatic vines had, often, but not always, greater proportions of necrotic wood than asymptomatic vines. With the same approach, Liminana *et al*. (2009) found positive correlation between the percentage of necrotic area within the wood of rootstock mother plants and the incidence of mortality in a collection of 11 rootstocks. Using rootstock cuttings inoculated with *P. chlamydospora*, Gramaje *et al.* (2010) found positive correlations between the percentage bud break and shoot weight of cuttings and disease severity, estimated by the vascular discolouration in the xylem vessels. Cutting transverse trunk sections, Maher *et al*. (2012) reported that vines with severe esca foliar symptoms showed an advanced stage of degradation of peripheral tissues. Calzarano and Di Marco (2007) found no correlation between the volume of degraded wood and incidence of leaf symptoms in one experiment, but a potential correlation in another. Lecomte *et al.* (2008) also reported that some individual symptomatic vines had lower proportions of necrotic area than asymptomatic ones. This indicates that the volume of deteriorated wood is a key-factor which is necessary, but not always alone sufficient, to explain the leaf symptom appearance. Thus, all factors that can favour increased proportion of inner wood necrosis should be investigated.

Esca is a disease characterized by summer foliar symptoms that precede the death of parts of vines or whole vines over variable time periods. Thus, two variables are often used, individually or together, to assess the damage of esca: the percentage of leaf-symptomatic vines and the percentage of trunk affected vines (dead vines and those showing spur or cordon death). Among the vineyards that were surveyed between 2004 and 2006 in the Aquitaine region, in a symptom study (Lecomte *et al*., 2012a), significant differences in disease incidence were noticeable between vineyards and also between both variables for individual vineyards. For example, a Lyre-trained vineyard showed the greatest incidence of vines expressing foliar symptoms, but had one of the least incidences of trunk-affected vines. The authors hypothesized that the length of the vine arms influenced the delay in vine decline. Travadon *et al*. (2016), comparing minimal- with spur-pruned vines, also showed that the pruning regime affected wood necrosis and esca incidence, with spur-pruned vines exhibiting more inner necrosis surface and being more affected than minimal-pruned vines.

Following the results obtained from the National surveys and those from all previous studies, our objective was to further investigate the role of cultural practices in development of GTDs, focusing on esca. Therefore, an additional survey was carried out in different French regions, by examining, in similar environments, comparable vineyard plots with different vine training and pruning practices with variable levels of esca damage.

Materials and methods

Survey

Two approaches were used to compare agronomic situations with different vine training or pruning systems: i) utilizing plots already established in experimental vineyards, and ii) identifying two vineyard plots located in the same environment (under soil and climatic conditions likely to be very similar), with the same cultivar, rootstock (where possible), vine vigour and age, and where one of the vineyards was known to have a high level of esca expression. The distance between two vineyard plots grouped in pairs did not exceed 800 m. Five vine-growing regions were surveyed from 2010 to 2016. These were: Bordeaux, Gers (Appellation Armagnac), Languedoc, Jura and Burgundy (Table 1). Vineyards were generally compared in pairs, but in one case (Gers) one vineyard was compared to two others.

Vineyards were selected to identify a training or pruning practice to be studied. Based on foliar symptoms, esca was always the most prevalent trunk disease in these plots. Vineyards with very low levels of damage but older than plots with high levels of damage were also selected. Because tillage may be an important alternative cause of vine death, all vineyards were non-cultivated between the vine rows. Vineyards with other potential sources of vine death were not included in the surveys.

The location and the main agronomic characteristics of the plots are described in Table 1, including the cultivars, rootstocks, training systems, distances between vines and rows and pruning regimes. Most

Table 1. Main cultural characteristics for the vineyard plots selected for the survey.

Viticultural area	Location	Vineyard name[a]	Cultivar	Rootstock	Date of planting	Training/pruning system and vine spacing intervals[b] (m)
Bordeaux (Gironde)	Latresne	*Grand Parc*	*Cabernet-Sauvignon*	*Gravesac*	*1997*	*'Espalier Guyot double'*;1.8 × 1.2
						'Cordon';1.8 × 1.2
	Saint Genis du Bois	*Lagrange 2*	*Merlot*	*5BB*	*1980*	*'Lyre'*;3.2 × 1.2
						'Espalier Guyot simple';3.2 × 1.2
		Lagrange 3	*Merlot*	*3309*	*1978*	*'Lyre'*;3.2 × 1.2
						'Espalier Guyot simple'; 3.2 × 1.2
		Lagrange 1	Cabernet-Sauvignon	SO4	1991	'Espalier Guyot simple';3.2 × 1.2
		Lagrange 4		101-14	1982	'Lyre';3.2 x 1.2
	Castillon	Castillon 1	Cabernet-Sauvignon	SO4	1990	'Espalier Guyot double';2 × 1.1
		Castillon 2	Cabernet franc		1991	'Espalier Guyot simple'; 2 × 1.1
Gers (Armagnac)	Gondrin	Gondrin 1	Colombard	SO4	1993	'Espalier Guyot simple'; 2.8 × 0.9
		Caubeyre		Gravesac	1996	'Espalier Cordon'; 2.8 × 1.1
		Gondrin 2		3309	1992	'Espalier Guyot simple'; 2.8 × 0.9
	Heux	Heux 1		SO4	1990	'Espalier Guyot simple'; 2.8 × 0.9
		Heux 2			1984	'Espalier Guyot double'; 2.8 × 0.9
Jura	Voiteur	Voiteur 1	Chardonnay	3309	1990	'Espalier Guyot simple'; 1.5 × 1
		Voiteur 2			1990	
Burgundy	Rully	Meix Cadot 1	Chardonnay	Unknown	1920	'Espalier Guyot-Poussard'; 1 ×1
		Meix Cadot 2		SO4	1980	'Espalier Guyot simple'; 1 × 1
	Channy	La Chaponnière		Unknown	1960	'Espalier Guyot-Poussard'; 1 × 1
		Saint Jacques		SO4	1991	'Espalier Guyot simple'; 1.1 × 1
Languedoc	Pouzolles	Prelong Olivier	Sauvignon blanc	SO4	1984	'Espalier Guyot-Lépine'; 2.5 × 1
		La Cayoutale			1984	Unpruned since 2011; 2.5 × 1
		Les Grèses			1985	'Espalier Cordon'; 2.9 × 1
		La Crouzette			1980	Unpruned since 2010; 3 × 0.9

[a] Italic font indicates vineyard plots originating from an existing experimental design. Adjoining plots belonging to one owner are underlined. Discontinued horizontal lines separate the plots compared in pairs (in Jura, one plot is compared to two others).
[b] Between and within row distances (m).

of the cultivars (Cabernet-Sauvignon, Cabernet franc, Chardonnay and Sauvignon blanc) are known to be susceptible to esca (Dubos, 2002; Bruez *et al.*, 2013).

Different training systems were compared: i) 'Espalier Guyot' forms with short versus long arms (as trunk extensions), with long arms usually pruned according to the 'Guyot double' system, which is considered less mutilating than the 'Guyot simple' system, with no or short arms (Gironde, Gers); ii) 'Espalier Guyot' forms with 'Espalier cordon' forms; or iii) with Lyre forms (Gironde), which is generally formed with arms containing three or four pruning zones far from the vine trunks.

Observations on local cultural pruning practices, such as the presence of large wounds or frequent changes of the main sap flow over the years, known to be favourable to esca (Lafon, 1921), were also noted. These were classified into three categories: "+" use of the same sap route over the years, pruning mainly 1-year- old canes and leaving wood stubs as long as possible past the top most buds, keeping large pruning wounds to a minimum; "+/-" some of the practices described above were not observed; or "-" use of different sap routes from one year to another and applying frequent large pruning wounds.

Disease assessments

Between 390 and 800 vines were examined in each vineyard. Except in Burgundy, all vineyard plots were surveyed for at least two consecutive years. Esca prevalence was assessed typically before harvest, in September or early October, and based on all external symptoms, as described by Lecomte *et al.* (2012a). Externally observed wood and foliar symptoms were assessed according to a severity index (Table 2) derived from previously described severity scales (Darrieutort and Lecomte, 2007; Lecomte *et al*., 2012a). From the field disease data, six variables per plot or replicate were calculated (Table 2). These were: 'V', the number of remaining original vines free of symptoms either in the wood (cankers, dead spurs or cordons) or in the foliage; 'I' (I = D + A + Y), the number of totally unproductive or young vines; 'E' (E = DA + R + U), the number of partially unproductive original vines; 'I+E', the total number of original vines with cordon or trunk damage; 'F' (F = S + APO1 + APO2), the number of vines with foliar damage, and the total number of affected vines; or 'T' (T = I + E + F). By construction, the variables 'I' and 'I+E' cannot decrease from one year to the next. Percentages of 'I', 'E', 'I+E', 'F' and 'T' were calculated based on the total number of original vines planted, and were used to present the esca impact for each plot. Chi-square tests ($P \leq 0.05$) were used to compare 2 × 2 distribution of plots with the four variables 'V', 'I', 'E' and 'F'. Additionally, variables 'I+E' and 'T' were used for variance analysis (Fisher-test ratio, $P \leq 0.05$) to compare plots originating from experimental designs.

Results

Twenty-five vineyard plots were surveyed, differing according to the training or pruning system (Table

Table 2. Esca disease severity index (Darrieutort and Lecomte, 2006; Lecomte *et al*., 2012a) used to assess the status of each original vine in the vineyards surveyed in this study.

Code	Vine condition
V	Original vine without any damage (leaves and wood)
S	Symptomatic vine showing foliar symptoms (whatever their severity)
APO1 - APO2	Vine showing a complete wilting on 1 or 2 arms (apoplexy)
DA	Vine showing a part of dead wood (often a dead cordon)*
R	Retrained or restored vine*
U	Vine trained with only one cordon (a dead cordon has been removed)
D	Dead vine*
A	Absent vine [a]
Y	Replanted, regrafted, marcotte, or young vine. (any vine planted after the planting date)

[a] Because esca was the commonly reported disease in the vineyards we surveyed, wood damage was assumed to be mostly caused by esca.

Table 3. Impacts of esca in the grapevine plots originating from the three field experiment in Gironde.

Plot	Trellising systems	Pruning practices	No. of vines surveyed	Year of survey	Unproductive and esca-affected vines (%)[a]					*F*-test ratio[b] for the variables	
					I	E	I+E	F	T	I+E	T
Grand Parc Latresne 4 repeats	'Espalier Guyot' Short arms 20-30 cm	+/-	640	2010	1.9	7.1	9.7	7.3	17	NS; F=3.229; P=0.169	NS; F=5.218; P=0.104
				2011	3.0	8.4	11.4	5.9	17.3	NS; F=5.218; P=0.104	NS; F=6.153; P=0.088
				2012	4.1	11.4	15.5	11.2	26.7	NS; F=5.721; P=0.095	NS; F=0.209; P=0.677
				2013	4.2	14.4	18.6	14.2	32.8	**S**; F=12.91; P=0.035	NS; F=8.490; P=0.060
				2014	5.2	17.3	22.5	10.1	32.7	**S**; F=33.61; P=0.008	NS; F=0.216; P=0.672
				2015	5.8	20	25.8	3.6	29.4	**S**; F=31.81; P=0.009	**S**; F=11.91; P=0.039
				2016	5.9	22.2	28.1	5.6	33.7	**S**; F=62.89; P=0.003	**S**; F=14.47; P=0.030
	'Espalier Cordon' Long arms 0-60 cm	+/-	640	2010	1.2	4.4	5.6	6.9	12.5		
				2011	2.1	4.2	6.3	4.2	10.5		
				2012	2.8	4.1	6.9	17.3	24.2		
			638	2013	2.8	4.1	6.9	14.6	21.5		
				2014	3.4	5.5	8.9	22.4	31.3		
				2015	3.4	7.7	11.1	6.1	17.2		
				2016	3.8	10.0	13.8	11.9	25.7		
Lagrange 2 Saint Genis du bois 3 repeats	'Espalier Guyot' Short arms 10-30 cm	+/-	482	2012	17.4	11.2	28.6	6.0	34.6	**S**; F=34.30; P=0.024	NS; F=6.891; P=0.119
				2013	19.3	14.1	33.4	5.4	38.8	**S**; F=29.98; P=0.028	NS; F=8.479; P=0.099
				2014	22	15.6	37.6	3.1	40.7	**S**; F=23.07; P=0.037	NS; F=8.376; P=0.101
	'Lyre' Long arms 40-80 cm	+/-	441	2012	8.2	4.5	12.7	10.9	23.6		
				2013	9.3	5.7	15.0	8.8	23.8		
				2014	10.4	6.6	17.0	7.9	24.9		
Lagrange 3 Saint Genis du Bois 2 repeats	'Espalier Guyot' Short arms 20-30 cm	+/-	665	2012	17.4	12.0	29.4	3.9	33.3	**S**; F=1008.3; P=0.000	**S**; F=70.62; P=0.010
				2013	20.3	12.2	32.5	7.2	39.7	**S**; F=515.2; P=0.001	**S**; F=6084; P=0.000
				2014	21.9	11.4	33.4	3.5	36.9	**S**; F=338.8; P=0.001	**S**; F=54.817; P=0.014
	'Lyre' Long arms 40-80 cm	+/-	686	2012	2.0	1.6	3.6	10.1	13.7		
				2013	2.8	2.0	4.8	10.9	15.7		
				2014	3.5	2.0	5.5	8.6	14.1		

[a] I = unproductive vines (I = D + A + CP); E = partially unproductive vines (E = DA + R + U); F = vines showing typical foliar symptoms (esca symptomatic vines).
[b] *Fisher*-test ratio resulting from variance analyses (in the table, *F* in italics is the value provided by the Fisher-test analysis). **S =** Fisher-test significant (grey highlighted cell). NS: Fisher-Test not significant. Results of plot pairs are compared year by year.

Table 4. Impacts of esca in two pairs of vineyards located in Gironde.

Plot	Main trellising system differences	Pruning practices	Year of survey	No. of vines surveyed	Unproductive and esca-affected vines (%)					Chi-Square test
					I	E	I+E	F	T	
Lagrange 1	'Espalier Guyot simple' Short arms (30-35 cm)	-	2014	579	40.4	33	73.4	1.0	74.4	S 1‰
			2015	579	45.6	30.9	76.5	1.2	77.7	S 1‰
Lagrange 4	'Lyre' Long arms (40-80 cm)	+/-	2014	655	16.5	25.2	41.7	16.5	58.2	
			2015	656	23.0	37.5	60.5	5.8	66.3	
Castillon 1	'Espalier Guyot double' Long arms (25-40 cm)	+	2011	600	2.0	6.2	8.2	0.2	8.4	S 1‰
			2014	600	2.5	7.2	9.7	1.7	11.4	S 1‰
			2015	600	2.8	9.0	11.8	1.2	13.0	S 1‰
Castillon 2	'Espalier Guyot simple' Short arms (15-25 cm)	-	2011	561	43.2	6.8	50	8.5	58.5	
			2014	561	52.6	20.2	72.8	3.4	76.2	
			2015	561	54.8	21.6	76.4	1.8	78.2	

1). These plots had 14 different owners, and allowed 13 comparisons of paired plots (Tables 3 to 8). Among these vineyard plot situations, six originated from three existing field experiments, with two to four replicates, located in the Gironde (Table 3). Among the comparisons of paired plots, eight comparisons were from the same farms [Latresne (one pair), St Genis du Bois (three pairs), Heux (one pair), Rully (one pair), or Pouzolles (two pairs)], suggesting similar vineyard practices for each pair. Apart from the rootstocks, in most plot comparisons, the cultivar, year of planting and soil-climate environments were identical or very similar. All assessments of esca damage were compared pair-wise by Chi-square tests, using the variables 'V', 'I', 'E' and 'F', and differences were statistically significant $P < 0.01$.

The percentages of cordon or trunk damaged vines ('I + E') generally increased year after year in all situations. Conversely foliar symptoms (variable 'F') often varied from year to year, confirming many similar observations in the literature. Disease symptoms were sometimes highly variable, as in the plot comparison 'Grand Parc at Latresne' (Table 3) with an F% range of 3.6 to 14.2 for the vines trained in 'Espalier Guyot', and 4.2 to 22.4 for the vines trained in 'Espalier cordon'. Consequently, the variable 'T' also varied in some years. Nevertheless, except for three plot comparisons (Tables 3 and 8), the variable 'T' also generally increased year after year. Thus, due to the reasons explained above, the variable 'I + E' was considered the most meaningful and is used hereafter in the comparisons and discussion of results.

Vineyards surveyed in Gironde

In the Bordeaux area, three pairs of plots within existing field experiments were examined (Table 3). Two different training systems were compared: an 'Espalier Guyot double or simple' form with short arms, versus an 'Espalier' form with long arms, either 'Espalier Cordon' or 'Lyre'. At Latresne, the 'Espalier cordon' trained vines were surveyed over seven consecutive years. The difference in terms of significant esca impact appeared in 2013 and were consistent for the variable 'I + E' up to 2016: vines trained in 'Espalier' cordons showed at least half the esca damage of the Guyot-trained vines. As an example, for 2013 to 2016, variance analyses carried out with the variable 'I + E' indicated a highly significant effect of the factor "trellising system" with F-test ratio = 62.89 ($P < 0.05$). In Saint Genis du Bois, the two other experimental trials also showed that vines trained in 'Espalier Guyot' were more dam-

Table 5. Impacts of esca in vineyards surveyed in the Gers department (three pairs of plots).

Plot	Training/pruning system	Pruning practices	Year of survey	No. of vines surveyed	Unproductive and esca-affected vines (%)					Chi-Square test
					I	E	I+E	F	T	
Gondrin 1	'Espalier Guyot simple' Short arms (10 cm)	-	2011	732	10.0	14.6	24.6	5.9	30.5	S 1‰
			2012	732	13.3	16.8	30.1	6.0	36.1	S 1‰
Caubeyre	'Espalier Cordon' Long arms (40-60 cm)	+/-	2011	758	7.8	6.9	14.7	3.8	18.5	
			2012	758	8.0	9.2	17.2	7.1	24.3	
Gondrin 1	'Espalier Guyot simple' Short arms (10 cm)	-	2013	746	17.6	20.9	38.5	1.9	40.4	S 1‰
			2014	746	19.2	21.2	40.4	7.9	48.3	S 1‰
			2015	696	23.6	19.8	43.4	5.7	49.1	S 1‰
			2016	752	25.9	21.7	47.6	3.7	51.3	S 1‰
Gondrin 2	'Espalier Guyot double' Long arms (80 cm)	+/-	2013	612	5.2	3.4	8.6	3.4	12.0	
			2014	612	6.2	2.9	9.1	10.8	19.9	
			2015	612	8.2	5.2	13.4	9.8	23.2	
			2016	612	10.5	5.9	16.4	16.8	33.2	
Heux 1	'Espalier Guyot simple' No or short arms (5 cm)	-	2014	800	32.3	5.5	37.8	9.5	47.3	S 1‰
			2015	800	32.9	9.9	42.8	8.0	50.8	S 1‰
			2016	800	39.0	5.4	44.4	7.6	52	S 1‰
Heux 2	'Espalier Guyot double' Long arms (40 cm)	+	2014	554	5.6	4.3	9.9	6.0	15.6	
			2015	550	7.6	8.9	16.5	2.9	19.4	
			2016	550	9.0	10.1	19.1	3.1	22.2	

aged by esca than the 'Lyre'-trained vines: at double the amount of disease in Lagrange 2 and six times more in Lagrange 3 ($P < 0.01$).

Table 4 showed results also collected in Gironde but from adjoining plots. Still in Saint-Genis du Bois, a third comparison of plots forming a pair and belonging to the same farm showed that the vines trained in 'Espalier Guyot' with short arms of Lagrange 1 were more damaged by esca than the 'Lyre'-trained vines of Lagrange 4. This difference was less important than in the experimental designs previously examined, which may be explained by the older age and poor pruning practices noted on vines of Lagrange 4. Conversely 'Lyre'-trained vines expressed more foliar symptoms. The other pair was close to Castillon (Table 6). Cabernet vines trained in 'Espalier Guyot' with long arms (Figure 1A) in the 'Castillon 1' plot were much less damaged by esca than Cabernet vines trained in 'Espalier Guyot' with very short arms (Figure 1D) in the 'Castillon 2' plot.

Vineyards surveyed in Gers

Three pairs of adjoining plots were in the department of Gers (Table 5). Plots trained according to the Guyot system with no or very short arms (Figure 1E), pruned according to the 'Simple Guyot' rules (one spur on one side and a long cane on the other), were all much more damaged by esca than plots with vines either trained in 'Espalier cordon' characterized by longer arms or Guyot-trained with long arms (Figure 1B) i.e. pruned according to the 'Double Guyot' rules (one long cane on each side). All comparisons of distributions were statistically significant ($P < 0.001$).

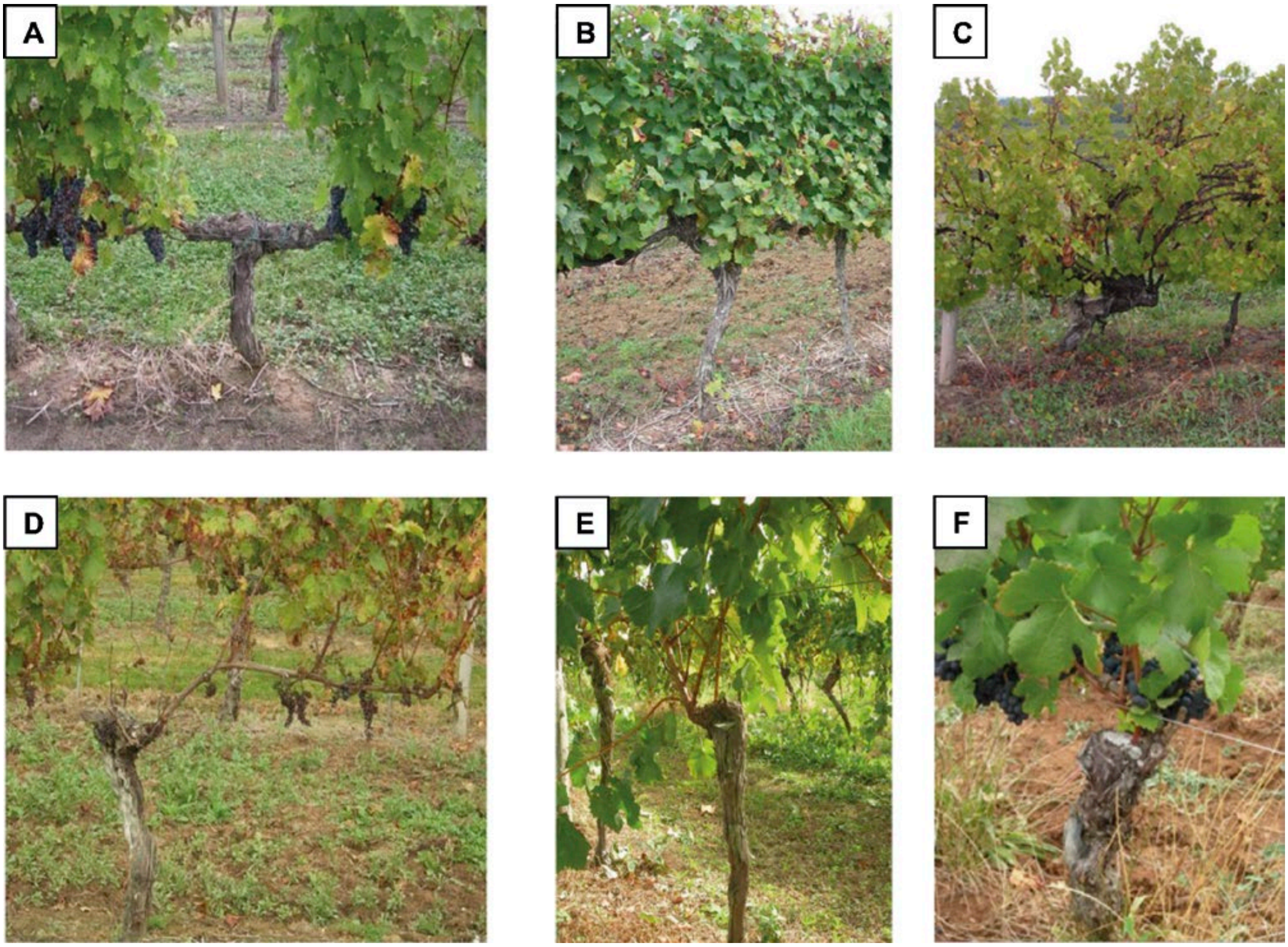

Figure 1. Examples of vines trained according to different trellising systems in France: on the top, those considered less conducive to esca (**A**, **B**, **C**); on the bottom, those considered very favorable for the disease (**D, E**, **F**). **A**, **B**: 'Espalier Guyot double', with long arms, examples with a Cabernet-Sauvignon vine in Gironde and with a Colombard vine in Gers.**C**: 'Espalier Guyot-Lépine' converted to minimal-pruned, example with a Sauvignon Blanc in Languedoc. **D**, **E**, **F**: 'Espalier Guyot simple', with no arm (or very short arms), and with either many pruning wounds concentrated close to the head of the vine trunk or a large pruning wound on the top of the trunk, examples with a Cabernet franc vine in Gironde, a Colombard vine in Gers, and a Pinot noir vine in Burgundy respectively.

Percentages of vines showing foliar symptoms varied greatly according to the year.

Vineyards surveyed in Jura

Two contiguous plots, belonging to different owners, were found and compared at the same location of Voiteur (Table 7). They were managed with similar training systems, with 'Espalier Guyot' pruning according to the 'Simple Guyot' rules with no or very short arms. However, the implementation of the pruning practices were quite different. Vines at 'Voiteur 2' were not conventionally pruned and showed large wounds due to severe pruning. The spurs and the canes selected when pruning were also chosen very close to each other and were often supported by the same sap route or wood sector. There were no significant differences in the observed foliar symptoms between both plots in 2015. In 2016, the 'Voiteur 2' plot had more foliar symptoms (6.6%) than the 'Voiteur 2' plot (0.2%). The total percentages of damaged vines by plot and by year for the these two years showed

Table 6. Impacts of esca in two adjoining vineyards located in the Jura department.

Plot	Training-pruning system	Pruning practices	Year of survey	No. of vines surveyed	Unproductive and esca-affected vines (%)					Chi-Square test
					I	E	I+E	F	T	
Voiteur 1	'Espalier Guyot simple'	+/-	2015	625	14.2	4.6	18.8	0.5	19.3	S 1‰
			2016	625	14.7	4.1	18.8	0.2	20	S 1‰
Voiteur 2	'Espalier Guyot simple' with large wounds	-	2015	603	32.1	6.8	38.9	0.2	39.1	
			2016	603	35.3	6.9	42.2	6.6	48.8	

Table 7. Impacts of esca in vineyards surveyed in 2016 in Burgundy (two pairs of plots).

Plot	Training-pruning system	Pruning practices	No of vines surveyed	Unproductive and esca-affected vines (%)					Chi-Square test
				I	E	I+E	F	T	
Meix Cadot 1	'Espalier Guyot mixte Poussard' Long arms (20-60 cm)	+	498	19.0	2.6	21.6	1	22.6	S 1‰
Meix Cadot 2	'Espalier Guyot simple'. Short arms (10-30 cm)	-	560	40.5	3.4	43.9	2.9	46.8	
La Chaponnière	'Espalier Guyot mixte Poussard' Long arms (20-40 cm)	+	618	30.1	0.3	30.4	0.2	30.6	S 1‰
Saint Jacques	'Espalier Guyot simple'. Short arms (10-20 cm)	-	568	49.8	2.1	51.9	2.9	54.8	

Table 8. Impacts of esca in vineyards surveyed in the Languedoc region (same farming system, two pairs of plots).

Plot	Training/pruning system	Pruning practices	Year of survey	No. of vines surveyed	Unproductive and esca-affected vines (%)					Chi-Square test
					I	E	I+E	F	T	
Prelong Olivier	'Espalier Guyot-Lépine'	+/-	2015	575	16.8	21.8	38.6	6.8	45.4	S 1‰
			2016		18.7	21.6	40.3	2.6	42.9	S 1‰
La Cayoutale	Not pruned since 4 vintages		2015	600	22	9.7	31.7	3.5	35.2	
			2016		22.3	10	32.3	0.3	32.6	
La Grèse	'Espalier Cordon'	+/-	2015	659	17.5	17.9	35.4	4.1	39.5	S 1‰
			2016		19.1	18.7	37.8	2.1	39.9	S 1‰
La Crouzette	Not pruned since 5 vintages		2015	600	24.2	4.5	28.7	0.8	29.5	
			2016		25	4.3	29.3	0	29.3	

Table 9. Basic and practical cultural measures methods for preventing rapid development of esca disease in grapevines. These have been prepared by François Dal (Sicavac), Laurence Geny-Denis (University of Bordeaux), Marco Simonit, Massimo Giudici and Tommaso Martignon (Simonit & Sirch), Jean-Philippe Roby and Lucia Guérin-Dubrana (Bordeaux Sciences Agro), and Barka Diarra and Pascal Lecomte (INRA UMR SAVE Bordeaux).

When planting and during the early period of vineyard growth	Prefer a training system and a vine density per ha that will allow formation of vine structures with long cordons and with pruning zones far away from the trunks
	Avoid early production, take time to well train the trunks and cordons before considering harvest. Remove the first grapes if necessary
	When pruning, never cut the canes close to the trunks and cordons, especially close to the graft point. Leave snags (to be removed the following year)
	Avoid large mutilating wounds as much as possible
After planting, during production stages	Prefer a pruning regime that will use the same sap routes over the years, according to the principles of the 'Guyot Poussard system' (Lafon, 1921), and avoid changes (inversions) of sap routes
	Prune as often as possible on young wood (1-year-old), and avoid pruning older wood
	Avoid large pruning wounds (electric pruning shears makes it easier to cut large branches and produce large wounds)
	Never prune close to the older wood to avoid dry and dead wood cones, especially along the trunks and cordons. Leave snags (to be removed the following year)
	In spring, anticipate the next winter pruning, during the disbudding or de-suckering periods, by leaving some shoots well positioned
	Follow the usually recommended prophylactic methods, particularly the removal of dead wood

that the 'Voiteur 1' vines were less than one half affected compared with the 'Voiteur 2' vines.

Vineyards surveyed in Burgundy

Two pairs of plots were selected close to the village of Rully in Burgundy (Table 7). In the first pair, Guyot-trained vines of 'Meix Cadot 1', with long arms pruned according to the 'Guyot mixte Poussard' rules and planted in 1920, were less damaged by esca and expressed less foliar symptoms than younger Guyot-trained vines of 'Meix Cadot 2', with short arms pruned according to the 'Simple Guyot' plot (Figure 1F). In the second pair of plots, Guyot-trained vines of the 'La Chaponnière' plot, with long arms pruned according to the 'Guyot mixte Poussard' rules and planted in 1960, were less damaged by esca and had fewer foliar symptoms than the younger Guyot-trained vines of the 'Saint Jacques' plot with short arms and planted in 1991.

Vineyards surveyed in Languedoc

Two pairs of plots were observed in this region within the same farm. The two main training systems in this area are the 'Espalier cordon' and a variant of 'Espalier Guyot', also called 'Lépine'. A new pruning system was been evaluated with two plots of Sauvignon blanc, a cultivar that is very susceptible to esca. These vineyard plots were converted to minimal pruned vines, respectively, 4 and 5 years before the first observation in 2015 (Table 8). Assessments of esca incidence in 2015 and 2016 showed that vines converted to minimal pruning (Figure 1C), in the 'La Cayoutale' and 'La Crouzette' plots, were less affected by esca and expressed less foliar symptom than Guyot-Lépine vines at 'Prelong Olivier' or cordons at 'La Grèse'. In 2016, no foliar symptoms were observed in the minimally pruned 'La Crouzette' plot. The differences of esca impacts within each pair of plots were highly significant ($P < 0.001$). However, because the Guyot-trained vines were converted to minimal pruning only for 4-5 vintages, the results indicated economically important responses: 32.6% of wood and leaf-affected for the 'La Cayoutale' vines in 2016, and 29.3% affected for the 'La Crouzette' plots. However, these disease impacts were not so evident for yield losses, because the leaf volume developed by minimal pruned vines filled the empty places due to dead or missing vines. An additional observation, taking into account only the empty spaces not filled by the minimally pruned vines, revealed (in 2016) a total of 20% of wood and leaf-affected for the minimally pruned vines of 'La Cayoutale', and only 12% for the similarly pruned 'La Crouzette' plots.

Discussion

The analyses reported here were from a survey of thirteen vineyard plot pair comparisons in France. Among theses, three were from established field experiments examining training systems, and located close to Bordeaux, while ten were from ajoining comparable plots located in different winegrowing regions. The results have supported previous conclusions for esca symptoms: vine training forms with longs arms (cordons) decline less rapidly due to the disease than forms with no or short arms, confirming previous observations of Lecomte *et al.* (2012b). In addition, the pruning decisions/options taken by winegrape growers also affected esca symptoms. These conclusions are supported by three major points: i), these trends are widespread in many regions; ii), the trends are supported by measurements in statistically designed and established field experiments, which are more stringent than other paired plot measurements; and iii), similar trends are documented in the literature (Geoffrion 1977; Bolay, 1979; Cordeau *et al*., 1984; Boubals et Mur, 1990; Dubos, 2002; Lecomte *et al.* 2008; Dumot, 2012).

Based on our observations of leaf symptoms, esca was typically the main trunk disease. However, this does not exclude the presence of other trunk diseases (Botryosphaeria, Eutypa, or Phomopsis diebacks) in the wood. Nonetheless, growers also acknowledged that esca was the main cause of vine death in these vineyards. However, it was not possible to affirm that all mortality and observations of dead cordons and spurs were due to esca. However, numerous previous reports also suggest the negative influence of intensive vineyard cultural practices on Eutypa dieback (Bolay, 1979; Cordeau *et al*., 1984; Boubals et Mur, 1990; Carter, 1991; Dumot, 2012).

The role of cultural practices, particularly that of pruning wounds, on the development of GTDs is reported to have been suggested since antiquity (Larignon, 2016). In France, to the best of our knowledge, the first indication of the role of severe pruning as predisposing vines to esca was by Abbot Rozier (1796). Grapevine is a liana, the wounds of which heal poorly, and it naturally grows by developing free forms with many branches. This relationship between severe pruning and grapevine dieback was also later reported by Dezeimeris in 1891, and in many other viticulture manuals (e.g. Dubos, 2002). To our knowledge, the first experiments on effects of pruning on esca were carried out by Eugène Poussard, a viticulturist in the Charentes area, whose observations and practices were recorded in a book written by René Lafon in 1921. Eugène Poussard is responsible for the vine pruning system presently called 'Guyot mixte Poussard', which is believed to significantly limit esca symptoms (Lafon, 1921; Dubos, 2002; Geoffrion and Renaudin, 2002; Dal *et al*., 2008; Lecomte *et al*., 2008). In Burgundy in the present study, where the 'Guyot mixte Poussard' system was practiced in two plots, there was less esca damage than in much younger 'Guyot simple' trained vines.

French viticultural literature contains many reports, albeit with little testing, on the role of cultural practices in esca (e.g. Geoffrion 1977, Bolay, 1979; Cordeau 1984; Boubals and Mur 1990; Dumot, 2007; Lecomte *et al*., 2008). Furthermore, we now know there are several distinct trunk diseases, the pathogens for which often occur in mixed infections together and/or with pathogens which cause esca. However, three main mechanisms have been highlighted by several authors (e.g. Lafon, 1921; Dal *et al*., 2008, 2013; Simonit, 2016): i), the number, location and size of pruning wounds in a restricted pruning zone on each vine; ii), the location, or change of location, of the main sap routes selected by the pruning system; and iii), pruning too close to the main trunk or cordon, which favours rapid development of dry necrotizing wood under the wound surface from the tissues exposed to the open air (Grosclaude, 1993). The worst pruning/training system is 'Guyot simple', as we noticed in all regions surveyed in this study. This vertical 'Espalier' system is based on a selection of one spur on one side each vine and of one cane on the other side, of a trunk with two arms. This often results in a form with two very short arms, and sometimes with no arms when the distance between vines in each row is small, and is exacerbated by close proximity of pruning wounds, especially of large diameter (Dal *et al*., 2008). The quantity of "necrotizing wood cones" in the same pruning zone close to the vine trunk may be more or less large or deep, and all will be proximal after several years, and these may interfere with the sap routes. Furthermore, if there is only one main sap route or wood sector to feed the foliage, or if there are frequent inversions or changes of the main sap routes or wood sectors (Lafon, 1921; Dal *et al*., 2008), this may favour the formation of non-functional wood in the trunk or arms. This may facilitate colonization by pathogenic or opportunistic fungi or other micro-organisms, as has been observed when severe pruning is carried out in urban horticulture (Carbonneau *et al*., 2015). The lack of skilled farm workers and the mechanization of pruning (favouring larger cuts) may contribute to the widespread occurrence of severe esca (Lecomte *et al*., 2011). Conversely, the 'Guyot mixte Poussard' pruning system, also based on a 'Guyot' form with one spur and one cane, avoids large wounds and minimizes interruptions of sap flow from one year to the next. These pruning rules, which are long forgotten until now, have been rediscovered in many regions with great success. This has been particularly influenced by pioneers such as François Dal, Marco Simonit and Marco Pierpaolo Sirch (Dal *et al*., 2008, 2013; Simonit, 2016).

In our survey, we compared different training systems which mostly varied according to the length of their cordons. All results showed that the longer were the vine arms, the slower was the development of dieback, as previously suggested by Lecomte *et al.* (2012a). Two explanations for this may be advanced: i), the longer the arms, the less rapid and less disruptive is the development of the inner necrosis; and ii), with a 'Guyot-simple' training system, there are only two main pruning zones, one on each side, and they are very close to the trunks, favouring rapid development of inner necrosis at the top of the trunk, which is quickly detrimental to vine survival. These explanations are supported by other findings: in Abruzzo, in central Italy, 40-year-old vineyards of the Trebbiano d'Abruzzo cultivar, trained to the expanded trellis system (e.g. "Tendone"), with trunks about 2 m high and with four permanent long branches, where esca was observed for 23 consecutive years, there was very low annual vine mortality and long-term vine survival, without visible wood damage (Calzarano *et al*., 2018). This confirmed the findings from the present study. Dumot *et al*. (2012) also found that 'Espalier Cordon' vines in Cognac were less affected by Eutypa dieback and esca than Guyot-trained vines. In the same way, vines trained in the Lyre system have several spurs or pruning zones. This form expresses foliar symptoms more often and for longer. With this form, however, it is very rare that entire vine arms suddenly die. Esca affects some spurs, but symptoms are restricted to a part of each affected vine. Furthermore, even if such spurs die, the sap route or wood sector under the arms remain functional for long periods (at least at the beginning of disease development). This is because the sap route or wood sector has been regularly used over the years. These characteristics may explain the longevity of arms of the Lyre form, and why Lyre-trained vines decline more slowly than forms with short or no arms and only two pruning zones.

We also examined 'Guyot-Lépine' vines, which were converted to unpruned vines (minimal pruning) in the Languedoc region. Results after four to five vintages, by comparison with classical 'Guyot-Lépine' vines, were very encouraging. Esca foliar symptoms were rare in minimally pruned vines. the absence of pruning the impacts of esca. Furthermore, in this type of training, vines growing into the places left by dead vines have increased foliage volume, and yield losses are reduced. Results on the effects of minimal pruning confirm those of Travadon *et al*. (2016), who showed that minimal pruning minimized the incidence of esca symptomatic vines and the percentage of vines with necrotic spurs or cordons, compared to 'Espalier cordon' trained vines. Dumot (personal communication) has also recently noted, from field trial results, that mechanical pruning, a system similar to minimal pruning (no wounds are made directly to the vine trunks or arms and only young canes are pruned), led to a significant reduction in esca compared to classical 'Espalier' trellising. Our findings suggest that severe pruning contributes to the development of esca symptoms. When sodium arsenite was still in use, this compound potentially masked the negative effects of severe pruning in some vineyards.

The present study raises other issues. One is that the assessment of esca impacts, only on the basis of foliar symptoms, is not sufficient. This is because of yearly fluctuations in disease expression and because esca affects grapevine wood. Many observers have noted foliar symptoms, and sometimes (but not always), the number of dead vines to assess esca impacts, without recording if there were visible wood necroses in cordons or spurs, and without observing if wood symptoms develop after the foliar symptoms. Only in some cases, with long arm trellising systems, losses in quantity and quality of the harvest were recorded despite the lack of externally visible wood damage (Calzarano *et al*., 2004). Damage to yield were also reported by Lorrain *et al*. (2012). Esca is often the main trunk disease encountered causing damage in most vineyards in European wine-producing regions. We suggest that the proportions of unproductive vines should also be recorded, as well as severity of leaf symptoms.

A second issue concerns the role of the pathogens. Our study clearly confirms that cultural practices are of significant importance in development of GTDs. The numbers of pruning wounds, their proximity, their ability to heal and their size, combined with frequent inversions of sap routes or changes of wood sectors, are likely to be major aggravating factors affecting the esca pathosystem. The data here reported and reviewed, suggest that the amount of dead and dry wood caused by training and pruning methods is very relevant for vine decline. The role of pathogens need to be better investigated in relation to cultural factors without neglecting their invasive roles. This assumption is supported by many studies indicating that inner wood necroses are associated with a large number of esca and GTD pathogens and saprobes, and these organisms are very common and present in most vines, whether leaf-symptomatic or not (Dumot, 2007; Lecomte *et al*., 2008; Liminana *et al*., 2009; Hofstetter *et al*., 2012; Travadon *et al*., 2016). All these results will encourage further research on vine physiology and hydraulic efficiency. This will allow greater understanding of the roles of inversions of sap route, frequent changes of wood sectors to supply the foliage, and more generally to better understand the effects of some pruning practices on development of esca and other GTDs. Table 9 shows some guidelines as basic and practical advice to prevent the rapid development of esca and, possibly, other GTDs. These suggestions might also lead to further studies, including experiments on the role of efficient pruning protection.

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