Impact of grafting type on Esca foliar symptoms

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Abstract:

Aim: The aim of the survey was to investigate if the grafting type influences the foliar expression of Esca during summer.

Methods and Results: Observations were conducted on plots distributed in two French winegrowing regions (two different climatic regions) with two varieties (Cabernet-Sauvignon in the Bordeaux region and Mourvèdre in Provence). Three grafting types were compared: Omega graft, Whip and Tongue graft, and full cleft graft. For both varieties, we found a significant effect of the type of grafting on the foliar development of Esca symptoms. Full cleft grafted plots showed a significantly lower percentage of Esca foliar symptoms than the other two modalities, which were not significantly different from one another. Concerning Omega grafted plots, a significant difference in the rate of Esca foliar symptoms was highlighted compared to full cleft grafted plots, with a higher rate on Omega grafted plots, but these plots were also younger.

Conclusions: The study established, for the first time, the difference between full cleft field grafted plots and Omega and Whip and Tongue grafted plots, revealing a higher incidence of Esca on the latter types of grafting.

Significance and impact of the study: The spread of mechanical graft could be one of the factors explaining the increasing incidence of Esca in vineyard.

Keywords: Esca, foliar symptoms, graft type, nurseries, quality of planting material

Introduction

Grapevine trunk diseases including Eutypa dieback, Esca and Botryosphaeria dieback are among the most destructive diseases affecting established vineyards. They have been reported in most winegrowing regions over the world and are responsible for loss of productivity and vine death. Since 1990, the incidence of Esca has increased drastically affecting nearly 10% of French vineyards (Kobès *et al*., 2005; Bruez *et al*., 2013).

These diseases attack the vine wood: they cause death of spurs, arms, cordons and sometimes entire vines upon wood colonization by various pathogens (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Surico *et al*., 2006; Van Niekerk *et al*., 2006; Lecomte *et al*., 2012). The development of necrosis inside the wood greatly hinders the sap flow and can induce a general weakening of the plant. The physiological balance of the plant seems affected when the critical volume of non-functional wood becomes too large (Lecomte *et al*., 2008; Luque *et al*., 2009; Maher *et al*., 2012). A high volume of inner necrosis may then seriously reduce water transport and may impact plant functioning, in particular in water stress conditions.

There are actually a large number of pathogens described associated with these diseases and increased knowledge about mechanisms of their development in vine wood (Bertsch *et al*., 2013). Furthermore, Esca is a complex syndrome (Mugnai *et al*., 1999; Graniti *et al*., 2000; Surico *et al*., 2006) and is still poorly understood unlike Eutypa dieback. The role of Botryosphaeria species in the development of the Esca syndrome is still a matter of debate (Mugnai *et al*., 1999; Lecomte *et al*., 2012). Both diseased and healthy adult plants show the same fungi species, suggesting they are normal mycota associated with adult vines (Hofstetter *et al*., 2012). These pathogens are latent and can become pathogenic under the influence of unidentified factors (Retief *et al*., 2006; Surico *et al*., 2004 and 2006).

There are no efficient methods for managing these diseases in the vineyard (Bertsch *et al*., 2009). Attempts to control these fungal diseases are currently based on the use of biological agents, natural molecules, chemical compounds and sanitation methods, alone or in combination (Darrieutort and Lecomte, 2007; Bertsch *et al*., 2013; Diaz and Latorre, 2013). Nevertheless, they are not yet completely effective and current research is focusing on the factors responsible for disease emergence (Lecomte *et al*., 2012).

Numerous factors are reportedly involved in the development of grapevine wood diseases (Lecomte *et al*., 2011; Hofstetter *et al*., 2012; Bruez *et al*., 2013). The quality of plant material and planting practices, vineyard management strategy and pruning systems are some factors that may influence the proportion of Esca symptoms in mature vineyards (Geoffrion and Renaudin, 2002; Lecomte *et al*., 2011 and 2012). It has been also shown for instance that young plants from nurseries already contain fungi associated with Esca and Botryosphaeria dieback (Larignon *et al*., 2007; Larignon *et al*., 2008; Aroca *et al*., 2010; Billones-Baaijens *et al*., 2013). Indeed, the quality of the initial plant material can promote the development of pathogens in grapevine wood. Graft quality is another important criterion for the global quality of plant. Grafted vines showed a higher percentage of symptomatic vines compared to own rooted vines (Andreini *et al*., 2014). Contrariwise, Fourie and Halleen (2006) showed that machine-grafted graft unions had lower pathogen incidences compared to hand-grafted graft unions in commercial nurseries. The authors explained these results by big grafting wounds created in hand grafting regime and by unsterile hands.

Plot age is also an important factor in the foliar development of Esca symptoms and has to be taken into account in comparative studies. Indeed, expression level is maximal between 15 and 35 years but high level of expression is now also found in several younger vineyards (Surico *et al*., 2006; Romanazzi *et al*., 2009). However, the National Grapevine Trunk Disease Survey conducted in France from 2003 to 2008 shows the incidence of Esca is maximal for vineyards aged between 15 and 25 years (Fussler *et al*., 2008; Grosman and Doublet, 2012).

Significant differences in Esca foliar symptom expression have been already recorded among grapevine cultivars, rootstocks, clones and pedo-climatic conditions (Surico *et al*., 2000; Marchi *et al*., 2006; Larignon *et al*., 2009; Kuntzmann *et al*., 2013; Travadon *et al*., 2013; Andreini *et al*., 2014; Murolo and Romanazzi, 2014). However, the incidence of Esca disease has highly increased over the past 25 years (Carbonneau *et al*., 2015). This period also corresponds to the generalization of the Omega grafting system. Grafting is an artificial multiplication technique which causes injuries and requires proper healing of plant tissue for good viability of future vines. Injuries or other wounds may favor the development of saprophytes or infections by trunk pathogens. Thus, another possible hypothesis is that the Omega grafting system might have promoted the development of Esca disease.

Omega grafting represents 95% of current grafting (http://www.vignevin-sudouest.com/publications/fiches-pratiques/production-plant-vigne-pepiniere.php). Grafting machines are used to cut and assemble the rootstock and the scion in a single manipulation. The mechanization of the grafting process is highly cost reducing. The Whip and Tongue grafting method is another grafting type examined in this study. The machine operates in two manipulations: double cutting in a Z shape followed by hand assembly (the traditional system with a low output per hour). For these last two graft types, grafting is performed in commercial nurseries according to standard practices. After matching the rootstock and the scion together, the newly grafted cuttings are packed in boxes and stacked in a humid, warm environment until the union has callused. This step seems to favor the transmission of fungi associated with Esca and Black dead arm (BDA) (Fourie and Halleen, 2006).

So far, little information exists on the role of the grafting method in the development of grapevine trunk diseases. Therefore, the purpose of our study was to investigate the putative influence of the grafting type on the foliar expression of Esca symptoms by comparing levels of Esca disease in different French vineyards where the three grafting systems described above were used to multiply the vines before planting.

Materials and methods

1. Grafting types

The study aimed at comparing the influence of one field grafting system, namely full cleft graft, and two table grafting systems, namely Omega graft and Whip and Tongue graft (Figure 1).

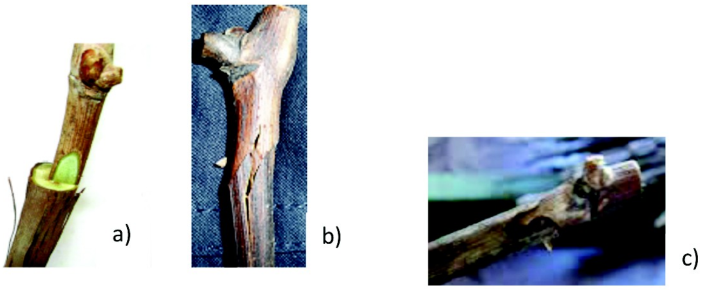

Figure 1. a) Full cleft graft, b) Whip and Tongue graft, c) Omega graft (Caroline Thienpont, 2013).

2. Network implementation

The study was carried out in two regions: southern France (Provence) and southwestern France (Bordeaux). These regions were chosen for their different climatic conditions, Mediterranean and oceanic, respectively. The studied varieties, Cabernet-Sauvignon in Bordeaux and Mourvèdre in Provence, are considered as sensitive varieties because they easily express foliar symptoms of Esca.

Couples or triplets of existing plots were selected in the same farm but with different grafting types, in order to avoid bias linked to vineyard management practices and/or pruning system. A total of 59 plots were monitored for two years. Plots were evenly distributed between the different grafting types and varieties, and the different types of grafts were equally represented in both regions (Table 1). The mean plot age was 47.1 ± 6.7, 41.3 ± 10.7 and 22.4 ± 4.5 for full cleft graft, Whip and Tongue graft and Omega graft, respectively.

Table 1. Number of plots used in this study per cultivar and per grafting type

Grapevine variety and region	Total number of plots surveyed	Graft Type	Number of plots per grafting type
Cabernet-Sauvignon Bordeaux area	19	Full cleft graft	5
		Whip and Tongue graft	7
		Omega graft	7
Mourvèdre Provence	30	Full cleft graft	10
		Whip and Tongue graft	9
		Omega graft	11

Observations

In each plot, all vines were assessed and assigned to seven categories: asymptomatic, showing mild summer foliar symptoms of Esca (also described as chronic form) or BDA (as described by Larignon *et al*., 2009), showing apoplexy, dead, missing or not original plant (re-planted or re-trained). A minimum of 300 original vines per plot which can express symptoms was considered necessary in order to be statistically representative. Finally, vines affected by either Esca or BDA foliar symptoms were not differentiated, i.e. named "Esca" in the rest of this publication. Esca observations were recorded only on plants dated from the year of planting. In each plot, percentages of Esca and apoplectic vines were calculated only on the basis of the number of original vines which can express symptoms (all original vines planted minus the dead, missing and re-planted vines). Observations were conducted in 2013 and 2014. In 2014, five plots (two full cleft grafted plots, two Whip and Tongue grafted plots and one Omega grafted plot) were not recorded because they had been uprooted.

3. Statistical analyses

The number of healthy vines and Esca affected vines was defined as a matched pair of counts. It was analyzed as proportion data using a GLM with binomial errors and logit link (Crawley, 2013). Overdispersion was checked by comparing residual deviance and residual degrees of freedom (R software; R Development Core Team 2010). When a significant effect of graft on Esca percentage was found, multiple comparisons were conducted to test differences between grafts using Tukey's HSD test.

Results

1. Esca and apoplexy rates

The percentage of plants showing Esca foliar symptoms slightly varied between years and varieties. Considering all grafting types, the mean percentage (% ± SE) of Esca is 5.30 ± 2.02 in 2013 and 5.36 ± 2.05 in 2014 on Cabernet-Sauvignon, and 5.11 ± 1.07 in 2013 and 4.39 ± 0.95 in 2014 on Mourvèdre.

The percentage of apoplectic symptoms of Esca was low. Whatever the grafting type, the mean percentage (% ± SE) of apoplectic form of Esca disease is 0.23 ± 0.12 in 2013 and 0.26 ± 0.12 in 2014 on Cabernet-Sauvignon, and 0.63 ± 0.18 in 2013 and 0.61 ± 0.18 in 2014 on Mourvèdre.

2. Grafting system effect on chronic form of Esca disease

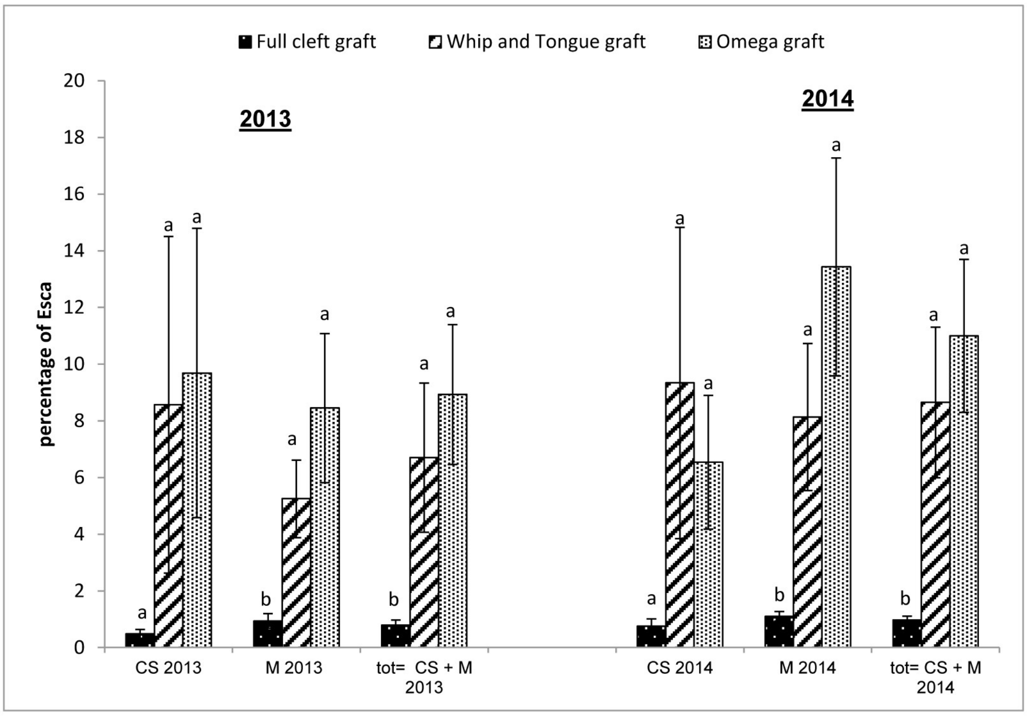

Figure 2. Mean (±sd) percentage of Esca vines per year and per variety ("Total" is the mean of Cabernet-Sauvignon (CS) and Mourvèdre (M) plots).

Analyses were carried out per year and per variety. Different letters above bars indicate significant differences between grafts (at $P < 0.05$).

Table 2. Results of GLM assessing effects of graft type on Esca percentage for the different years and varieties.

For each test, all df=2, 3 different grafting types (FC = Full cleft graft; WT = Whip and Tongue graft; O = Omega graft). Grafting types are ranked (>: significant difference between grafts; ≥: value is higher but not significantly different).

Year	Variety	LR Chisq (df)	P	
2013	Cabernet-Sauvignon	2.7 (2)	0.27	FC≤WT≤O
	Mourvèdre	16.9 (2)	0.0002	FC<O≤WT
	Total	18.2 (2)	0.0001	FC<O≤WT
2014	Cabernet-Sauvignon	4.6 (2)	0.1	FC<O≤WT
	Mourvèdre	14.7 (2)	0.0006	FC<WT≤O
	Total	19.1 (2)	< 0.0001	FC<WT≤O

For both years and both varieties, there was a significant effect of grafting type on the rate of chronic form of Esca (Figure 2). Full cleft grafted plots showed significantly lower percentages of Esca foliar symptoms than Omega or Whip and Tongue grafted plots (Figure 2). Thus, full cleft grafted plots showed percentages of Esca 6 to 15 times lower than the other two types of grafts for each year and grape variety considered.

On Cabernet-Sauvignon, for both years, full cleft grafted plots showed less vines with Esca symptoms than both other types of graft plots. In 2013 and 2014, on Mourvèdre, we found significantly lower percentages of Esca symptoms on full cleft grafted plots compared to Whip and Tongue grafted plots and Omega grafted plots (Table 2).

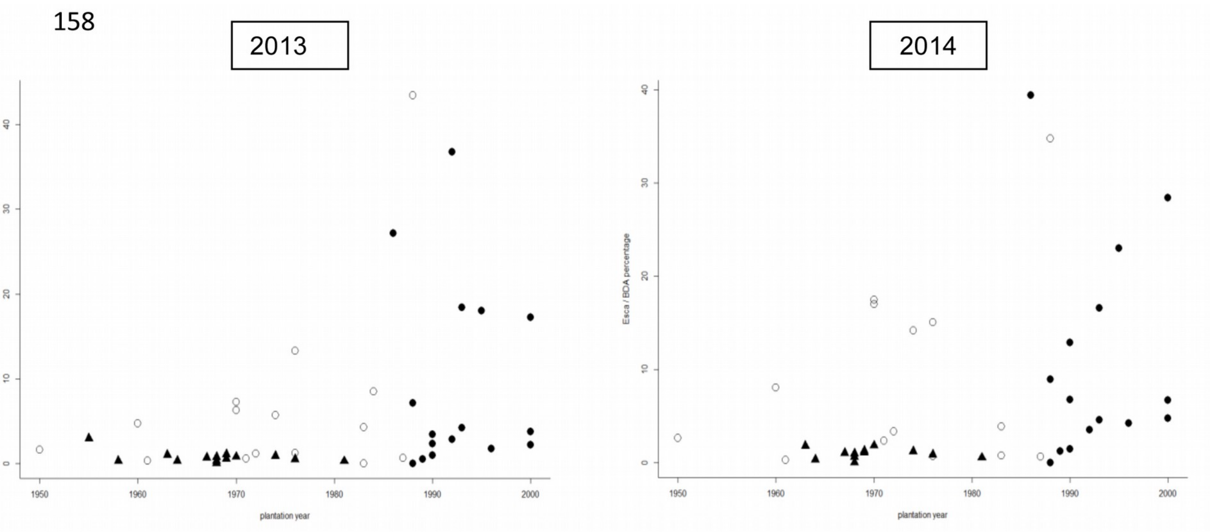

Figure 3. Percentage of Esca symptoms of plots according to date of planting.

Triangles represent full cleft grafted plots, open dots represent Whip and Tongue grafted plots and closed dots represent Omega grafted plots).

Figure 3 represents the percentage of Esca symptoms as a function of planting year and highlights the age difference among plots. Omega grafted plots were younger than the two others, whereas the years of planting of the full cleft grafted plots and Whip and Tongue grafted plots spread out over the same period. Therefore, it appears that only the comparison between full cleft grafted plots and Whip and Tongue grafted plots is rationally possible. On the other hand, the comparison between these two graft types and Omega grafted plots was biased because of the younger age of the latter plots. However, the rates of Esca of Omega grafted plots appear to reach the same orders of magnitude as the rates of Whip and Tongue grafted plots and are higher to the rates of full cleft grafted plots.

3. Grafting system effect on apoplectic form of Esca disease

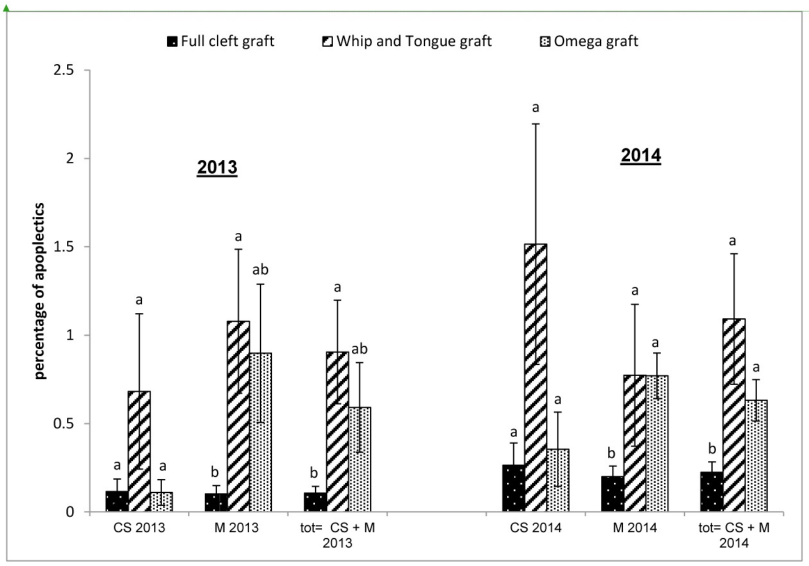

Figure 4. Mean (±SE) percentage of apoplectic vines per year and per variety ("Total" is the mean of Cabernet-Sauvignon (CS) and Mourvèdre (M) plots).

Analyses were carried out per year and per variety. Different letters above bars indicate significant differences between grafts (at P < 0.05).

Table 3. Results of GLM assessing effects of graft type on apoplectic percentage for the different years and varieties. "Total" corresponds to the analyses of the mean of Cabernet-Sauvignon and Mourvèdre plots.

For each test, all df=2, for 3 different grafting types (FC = Full cleft graft; WT = Whip and Tongue graft; O = Omega graft). Grafting types are ranked (>: significant difference between grafts; ≥: value is higher but not significantly different).

For both years and both varieties, a significant effect of the grafting type was found, with a lower percentage of apoplectic vines for full cleft grafted plots compared to both Omega and Whip and Tongue grafted plots. The percentages of apoplectic vines in Omega grafted plots were higher than those observed in full cleft grafted plots but lower than those observed in Whip and Tongue grafted plots. A similar pattern was observed in both years but the difference was significant only in 2014 (Figure 4).

When the two varieties were analyzed separately (and associated climatic regions), results were quite similar. On Cabernet-Sauvignon, no significant difference was found between grafting types in 2013 and in 2014, but Whip and Tongue grafted plots showed a higher rate of apoplectic vines compared to the other two graft types and for both years (Table 3). On Mourvèdre, in 2013 and 2014, full cleft grafted plots showed a lower rate of apoplectic vines than Whip and Tongue grafted plots (Table 3). A significant difference was found between full cleft grafted plots and Omega grafted plots in 2014. The same trend was observed in 2013 (although not statistically significant), with less apoplectic vines for full cleft field grafted plots (Table 3).

Apoplectic	Variety	LR Chisq (df)	P	
2013	Cabernet-Sauvignon	3.9 (2)	0.14	O≤FC≤WT FC=O=WT
	Mourvèdre	9.0 (2)	0.01 *	FC<WT≥O FC=O=WT but FC<WT
	Total	9.8 (2)	0.007 **	FC=O=WT but FC<WT
2014	Cabernet-Sauvignon	6.1 (2)	0.05 *	FC=O=WT
	Mourvèdre	9.6 (2)	0.008 **	FC<O=WT
	Total	12.1 (2)	0.002 **	FC<O=WT

Discussion

The mean percentages of vines showing apoplectic or chronic forms of Esca, whatever the grafting type, were consistent with those observed by the French National Grapevine Trunk Disease Survey (Fussler *et al*., 2008; Bruez *et al*., 2013). Furthermore, our survey, conducted in two representative French winegrowing regions, showed a weak variation of foliar expression between years. In both regions, high differences have been observed between plots (0% to 43% of vines with Esca foliar symptoms), particularly on Omega (0% to 39.9%) and Whip and Tongue grafted plots (0% to 43.4%). This observation suggests there is a difference in Esca foliar expression due to the quality of the batches of plants. As shown in Tuscany, when there is a substantial increase in the demand for propagating material, and hence in the production of grafted rooted cuttings, there is probably a decrease in plant quality (Surico *et al*., 2004). The authors highlighted the possibility that nurseries were forced to produce as much plant material as they could, of whatever type, including almost certainly shoots from Esca-infected vines. Several studies showed canes of rootstock mother were still infected by *Phaeomoniella chlamydospora* (Retief *et al*., 2006).

Whatever the grape variety, results showed a lower percentage of apoplexy on full cleft grafted plots compared to Whip and Tongue and Omega grafted plots. Whip and Tongue grafted plots showed the highest rates of apoplectic plants. Omega grafted plots exhibited intermediate rates of apoplectics with no significant difference with Whip and Tongue grafted plots.

The percentage of vines showing a chronic form of Esca was not significantly different between Whip and Tongue grafted plots and Omega grafted plots (6.70% and 8.93%, respectively, in 2013 and 8.65% and 11%, respectively, in 2014). However, in this survey, these two grafting systems showed significantly higher rates of chronic form of Esca compared to full cleft grafted plots realized on field. Indeed, the rate of Esca foliar symptoms on this grafting system was below 1% in both 2013 and 2014. However, in the Bordeaux region and Cabernet-Sauvignon variety, we did not notice strong and significant differences between graft types because of high variation in the percentages of Esca affected vines between plots. But the trend remains the same as Mourvèdre with a lower rate of Esca on full cleft grafted plots and a higher rate on Omega grafted plots.

Due to the recent development of this industrial technology, the Omega grafted plots were younger than the other two types of graft plots. Indeed, Omega graft was invented in the 1980s' and this graft type is related to vine age. The age of the full cleft grafted plots and Whip and Tongue grafted plots was comparable, with mean age (year ± SE) of 47.1 ± 6.7 and 41.3 ± 10.7, respectively. Omega plots were younger (22.4 ± 4.5). This age difference between Omega grafted plots and the two others may induce a bias in the expression of Esca rates. Indeed, expression of Esca foliar symptoms varies with the plot age. In this survey, Omega grafted plots were slightly younger than the maximal period of Esca expression (Surico *et al*., 2006) and in the maximal period shown in the National Grapevine Trunk Disease Survey (Fussler *et al*., 2008). Thus, the rates on these plots may decrease as plots become older. Further studies may focus on older Omega grafted plots to allow a better comparison of this more recent technology.

However, other factors could be involved such as the quality of material or plantation. Environmental and plant material factors have been much studied in the last ten years. Impacts of rootstock, climate and training system on Esca foliar symptoms were established (Surico *et al*., 2000; Marchi *et al*., 2006; Boso *et al*., 2008; Larignon *et al*., 2009; Van Niekerk *et al*., 2011; Andreini *et al*., 2014; Murolo and Romanazzi, 2014; Spagnolo *et al*., 2014). Impacts of planting conditions need to be taken into account. In Mediterranean conditions, spring is warmer than in oceanic conditions. Vine growers usually plant their vines earlier in the season than those from oceanic conditions where spring is usually wetter. These differences in planting conditions could affect vine development and sensitivity to Esca foliar symptoms. To take account of a maximum of factors, further studies may also carry out a trial with all grafting systems on the same plot, in the same planting conditions.

Significant differences in the percentage of Esca symptoms were revealed in this survey between the other two graft types: full cleft field grafted plots showed fewer foliar symptoms than Whip and Tongue grafted plots. Several studies showed a high rate of fungi contamination occurring along the propagation process in nurseries (hydration, disbudding, callusing and rooting, etc.) (Fourie and Halleen, 2006; Larignon *et al*., 2009; Aroca *et al*., 2010; Gramaje and Armengol, 2011; Agusti-Brisach *et al*., 2013; Billones-Baaijens *et al*., 2013). Full cleft grafted vines are not subject to these operations. Manual grafting in the field avoiding the nursery environment reduces potential contacts with fungi and finally shows lower rates of Esca foliar symptoms. Furthermore, the summer period is less favorable to spore dissemination of *Phaeomoniella chlamydospora*, which is considered as a pioneer fungus (Larignon *et al*., 2009; Bertsch *et al*., 2013).

Another explanation could be that greater surface area of cambium contact between rootstock and scion would assure a better graft quality. In order for the grafting operation to be successful, the vascular cambiums responsible for cell division of the two grafting partners must be in contact with each other so that they can build a connection between their separate vascular systems for water and nutrient supply (Keller, 2010). At each cut edge of the two cambiums, the callus, a mass of undifferentiated cells, grows and finally the scion becomes a part of the whole plant vascular system. Traditionally, grafting was carried out by hand and on one- or two-year-old rootstocks. This manual on-field grafting (or full cleft graft) promotes greater surface contact between cambium compared to mechanical on-table grafting (Omega graft and Whip and Tongue graft).

Moreover, when the rootstock is rooted, the plant focuses all resources on development of continuity between the two plant vascular systems. Plants grafted in field already have an established root system and consequently are more resistant and allocate more resources to callus production. Table grafted plants have to allocate some resources to root production and therefore fewer resources to establish the callus and the relation between the two vascular systems of rootstock and scion. Fourie and Halleen (2006) found more pathogens on machine-grafted graft unions compared to hand-grafted graft unions. These results are not contradictory because more pathogens are not always associated with greater incidence of Esca disease. Hofstetter *et al*. (2012) indeed showed that healthy asymptomatic vines carried the same fungi as diseased vines (Hofstetter.

Conclusion

As a conclusion, in this study, Whip and Tongue grafted plots show higher percentages of apoplexy and chronic form of Esca compared to full cleft field grafted plots. Several hypotheses can explain these results. First, the nurseries are a high source of fungi contamination. Then, the contact surface between cambiums may be different according to graft type. Finally, vines grafted manually on field are already rooted, which means that they could allocate more resources to callus production.

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