

Impact of biotic and abiotic factors on the development of Esca decline disease

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Abstract: The aim of researches performed by our group is to determine which internal and external factors have a key influence on grapevine wood diseases, especially on Esca. The relationship between Esca leaf symptoms, the size of inner necrosis, the fungal endophytes associated with the disease and the whole microflora that can be detected from the grapevine wood, is discussed. Recent studies on leaf symptom development showed that summer temperature conditions likely play a major role in the expression of Esca symptoms. Cultural factors, like plant vigor or type of soils, also have presumably a significant influence. Several evidences suggest that other abiotic factors linked to the training systems (e.g. forms with very short cordons) or to pruning decisions (e.g. winter hand pruning characteristic of the modern grape-growing training systems) have also to be taken into account, alone or combined with other factors. Whole data indicate that Esca is a multi-factor disease and that many complex pathological scenarios could explain the grapevine trunk disease expression. A control strategy, mainly based on cultural measures to avoid a too early development of inner necrosis, is proposed.

Key words: aetiology, cultural preventive measures, integrated control, prophylaxis

Introduction

Esca is a worldwide syndrome associated with the development of fungal endophytes into the grapevine wood. This major trunk decline disease is found in all European vine-growing areas and lead to entire or partial decline of vines. In France, the National Grapevine Wood Disease Survey has recently reported that the percent of no-productive vines affected by vine trunk diseases (including *Eutypa dieback*) is about 10% (Grosman, 2008). In the last decade, as the incidence of Esca or Bot cankers seemed to increase regularly in some regions and to appear earlier and more severely in some vineyards, it was even assumed that these grapevine trunk syndromes were emerging diseases. This context has encouraged more investigations. Based on studies in progress or on their own experience, authors of the current paper briefly review the main biotic or abiotic factors that may have an influence on grapevine trunk disease development, particularly on Esca. The final objective is to suggest cultural methods for the preventive control of these insidious diseases.

Biotic factors

Leaf symptoms of Esca are known to be highly variable (Surico *et al.*, 2006), both in their incidence and in their shape (Lecomte *et al.*, 2006). They concern leaves with various and evolutionary discolorations, interveinal necrosis and wilting of branches and include those

attributed to Black Dead arm (Lecomte *et al.*, 2006, 2008a). The most dramatic symptom is the sudden wilting of entire vines, also called apoplexy. Mild symptoms appear sporadically in most of the vineyards (Mugnai *et al.*, 1999). Leaf symptoms of Esca are generally associated with the presence of wood necrosis and decays into arms and trunks. In French vineyards, the mean percentage of vines showing Esca leaf symptoms is around 4% (Kobès *et al.*, 2006). However, the mean percentage of vines with inner necrosis may reach 100% in some vineyards or mother vines (Dumot *et al.*, 2007; Lecomte *et al.*, 2008; Liminana *et al.*, 2009). This difference between the high level of vines affected by inner necrosis development and those of vines exhibiting leaf symptoms is still a matter of questioning. To better understand the relationship between inner necrosis and leaf expression, various studies have been carried out with vines collected in Bordeaux area. Preliminary results (Guérin-Dubrana *et al.*, 2008; Lecomte *et al.*, 2008b) pointed out that the type of necrosis and/or their size, assessed by image analysis, is correlated with the leaf damages. However, as reported by Calzarano and Di Marco in Italy (2007), the necrosis size is likely not always sufficient to explain the variability of leaf expression of the Esca disease. Many other factors like plant defence efficacy (Gaudillère, 2003; Goutouly, 2007) or the microbial activity are also assumed to play a significant role in the leaf symptom occurrence.

Isolations of fungi carried out from Esca-affected trunks showed that necrosis were usually colonised by the same fungal pathogens that can act as wood-degrading agents (Fisher and Kassemeyer, 2003; Larignon & Dubos, 1997; Péros *et al.*, 2008). The aggressiveness of these pathogenic fungi was recently investigated (Laveau *et al.*, 2009) based on pathogenic tests performed into the wood of young cuttings. Results confirmed that all the fungi studied can be associated with necrosis and two of them, *Phaeoconiella chlamydospora* and *Neofusicoccum parvum* developed significant canker lesions and inner necrosis. Nevertheless, only one fungus, *Eutypa lata*, up to now, is capable to provide necrosis and more or less regularly leaf symptoms using this bioassay (Péros & Berger, 1994). Several studies indicate that toxins are likely involved (Sparapano *et al.*, 2000). However, no complete demonstration of the Koch's postulate have been obtained after inoculation of plants by the fungi putatively involved (Sparapano *et al.*, 2001), indicating that conditions inducing the foliar disease expression in the summer remain unclear.

Gubler *et al.* (2005) reported that more than 1300 different species of fungi or bacteria were found to colonize grapevine wounds in spring. There is still a lack of information concerning the role and the activity of the whole microflora colonizing or living into the grapevine wood, either necrotic or apparently healthy. Preliminary results of isolations on culture media done from 2006 (data not shown) in different Bordeaux vineyards also revealed that wood inhabiting parasites can be very diverse, including bacteria and yeast. Therefore the whole microflora that can be identified from the grapevine wood is probably not yet determined. To know more, especially about the inoculum pressure in the apparently healthy wood, we are presently characterizing the trunk-colonizing microflora by cultivable and molecular methods, i.e. the single-strand conformation polymorphism (SSCP) fingerprinting technique and microbial DNA-sequencing. The main advantages of SSCP are that it can be used to detect rapid changes in microbial communities in the absence of prior knowledge about their composition and it avoids the biases introduced by culture-based methods.

Abiotic factors

In France, local surveys showed that the disease expression can reach percentages higher than 20% or much more in some vineyards (Lecomte *et al.*, 2005; 2008b). Foliar expression, depend on the years, the vineyard areas, the vine-age and the varietal susceptibility factors already well-known (Dubos, 2002; Fussler *et al.*, 2008). Various factors may explain the

variability of Esca incidence and its sporadic character (Mugnai *et al.* 1999). Among them, climate is often the first abiotic factor cited to explain variable disease incidences between years or between areas. Surico *et al.* (2000) did not detect in Italy (Florence and Siena) any weather condition conducive to Esca, nevertheless rainy summer was even so found more favourable to the chronic form meanwhile dry summer was found more conducive to the acute form. In France, apoplexy is known to often occur after a rain in a warm period (Dubos, 2002; Galet, 1995). Marchi *et al.* (2006) tended to confirm that rainfall seems positively related with manifest Esca. However, in spite of climatic variations may easily explain variable levels of disease expression or disease rates, preliminary results of a survey done from 2004 to 2007 in vineyards from Aquitaine region (Lecomte *et al.*, 2006, 2008 a,b), showed that the appearance of leaf symptoms increased more or less progressively and regularly from the beginning of June up to the end of July (see an example in Figure 1). After this date, the rate of leaf symptom occurrence decreases, although symptoms may occur up to September. This kind of profile for Esca symptom appearance was observed whatever the vineyard and the year and was also suggested by Marchi *et al.*, 2006. Such a profile tends to indicate that the progressive appearance of vines showing leaf symptoms could be strongly related to the progressive increase of mean temperatures in early summer (Darrieutort *et al.*, 2007), which likely influence the fungal growth or activity in the wood tissues.

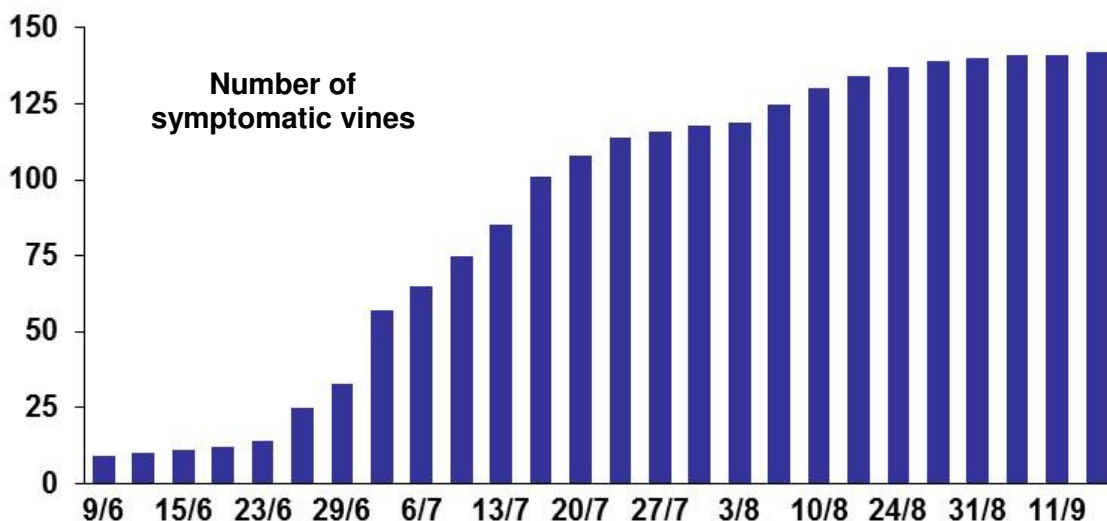


Figure 1: Example of Esca leaf symptom occurrence during summer recorded in a vineyard located in Bordeaux area (C nac, France, 2006).

Soil may also play a significant role in the development of Esca symptoms. A survey of 22 plots representative of Bordeaux area was done in summer 2004-2006 to look for a relationship between leaf expression and the main soil characteristics (texture, water availability and fertilizer). In preliminary results, Guerin-Dubrana *et al.* (2005) reported that the soil with high percentage of lemon and clay and with either high water reserve or with high nitrogen supply were the most conducive to Esca. These first results indicated a link between the disease and the vigor and/or the growth conditions. This influence of the soil, especially the slope, was also partly reported by Surico *et al.* (2000), that was more conducive when the slope was more level. Robotic and Bosancic (2007) reported exactly the same result

in a vineyard from Serbia. Panon *et al.* (2005) also reported higher disease incidence in the heavier soils of Champagne.

Information concerning the influence of training or pruning systems are still fragmentary and sometimes controversial. Most of the reports were found in the French technical literature from the last century. However, because all fungi associated with trunk diseases can enter the grapevine wood through the pruning wounds, many authors tended to point out that the number of pruning wounds, their size, their position and their concentration in the same pruning zone are key-factors that can lead to a quick development of inner necrosis (Bolay, 1979; Boubals and Mur, 1990; Dubos, 2002; Lecomte *et al.*, 2008). One of the first study carried out about the influence of a training system, was the one performed by Lafon in 1927 who developed a pruning system previously used by a grower settled in Charentes, Mr Poussard. The principle consists in keeping the same sap route from one year to another. Wounds are on a same line and are only made on the upper part of the cordons. This system, called Guyot-Poussard, was found less conducive to Esca by Geoffrion et Renaudin (2002) in the Loire Valley and is today recommended in regions where the “Guyot” form is usual.

Grapevine is botanically a creeper that naturally run the ground and can grow up along the trunks of other perennial plants. Current training systems often consist on developing a small fruit tree involving every year repeated pruning decisions (Lecomte et Gaudillère, 2008). This kind of training-pruning system is rather specific to viticulture, quite different from those used for fruit growing. This system is particularly severe and may have consequences on the development of inner necrosis, as exemplified by Table 1 showing that the percentages of necrotic surface, observed from transverse sections of vines, cut before uprooting, may vary considerably according to the training plus pruning systems. Moreover, wounds of the cultivated grapevine species do not heal well and short-cut pruning wounds are currently strongly unwise to avoid a too rapid drying and necrosing wood along the sap routes.

Table 1. Examples of inner necrosis development according to the pruning regime. Each necrotic surface was visually assessed on transverse trunk sections cut just below the arms of 22 Cabernet Franc vines per treatment.

Training system 2278 vines/ha 20 yrs-old	Pruning system from 1990 to 2004	Mean % of necrotic surface per transverse cut	% of vines with a necrotic area of more than 5% of the total trunk surface
Lyra: opened	Winter pruning	20.2 ^a	84 (S*)
Lyra: inversed	Winter pruning	13.3 ^a	41 (S)
Lyra: inversed	Minimal pruning in summer	2.3 ^b	9 (S)

^{a, b}, letters indicate significant differences following a Newmans-Keuls test ($\alpha=5\%$) and ANOVA testing.

S* Significant differences between treatments after a X^2 test carried out with a distribution of necrotic surfaces in 2 classes (upper or lower than 5%). Each treatment was different from the 2 others.

Since the 1990's, demand for planting material has probably exceeded supply of high quality plant material (Waite and Morton, 2007). Grapevine has also become a speculative crop and many current plantings are produced earlier than previous ones some decades ago. A new

syndrome described as Petri disease has been identified. And very simplified training systems have also emerged like those with trunks with low diameter and very short cordons, as shown in Figure 2, leading to a concentration of large pruning wounds close to the upper part of the trunks. This kind of training system, sometimes encountered in some vineyards with high yield potential was already considered as disastrous for Eutypa dieback. In “Entre-Deux-Mers”, a viticultural Bordeaux region, where highly contrasted situations can be noticed as far as incidence of either Eutypa or Esca is concerned, a tentative restricted survey was done in two representative locations in order to look for putative relationship between the different agronomical characteristics and the global incidence of Esca (Table 2). This etiological approach showed important differences between plots from the same location and revealed that the differences of Esca incidence could be explained by a set of favourable factors (susceptible cultivar, intrinsic rootstock vigour, short cordons, fertilizer, plant vigour due to environment, topography, ...).

Table 2. Examples of highly variable damage caused by Esca in different agronomical contexts. Survey done in Bordeaux area in 2007 (Adar Créon-Cadillac- CA33).

Location	Cultivar Rootstock Planting year	Training system Arm length Trunk height	Fertilizer N, K	Other characteristics	Sanitary status**
Tresses	Cab. Sauvignon S04 1990	Guyot 5-30 cm 80 cm	N: 21 Unit/an	High vigor*: 111.1g Humid plot, lower part Previous crop: grass-land 2666 vines/ha	360 89,8%
	Cab. Sauvignon 101-14 1983	Guyot 30-40 cm 60-80 cm	N: 21 Unit/an	Moderate vigor: 102.9g Sloping and draining soil Previous crop: grapevine 3333 vines/ha	172 40,1%
	Cab. Sauvignon 101-14 1987	Guyot 5-30 cm 80 cm	N: 21 Unit/an	Moderate vigor: 87.8g Soil with lemon and clay Previous crop: grapevine 2666 vines/ha	273 57,7%
Bonnetan	Cab. Franc 420A 1991	Guyot 5-15 cm 80 cm	K: 120 Unit/an	Moderate vigor: 86.5g Previous crop: grapevine 3333 pieds/ha	298 65,2 %
	Cab. Franc 3309 1984	Guyot 20-30 cm 60-70cm	No one since 2002	Moderate vigor: 71.2g Previous crop: grapevine 4545 vines/ha	67 23,4%

* Vigor visually assessed and measured by the dry weight of 100 leaves collected on September 2007

** Whole vines Esca-affected: vines with dead arms or entirely dead, re-trained, re-planted and symptomatic



Figure 2. Examples of simplified training systems that seemed very conducive to Esca in some French vineyards as reported in Table 1 (Photographs Gaudillère and Darrieutort)

Table 3. Control measures recommended for preventing trunk decline diseases, especially for Esca

Before planting	<ul style="list-style-type: none"> Use controlled mother-vineyards (age limited) Use plant material of good quality Avoid the most susceptible cultivars in the most fertile soils
Planting	<ul style="list-style-type: none"> Avoid too long immersions of roots in water Avoid too late plantings (July, ...) or provide appropriated irrigation
After planting	<ul style="list-style-type: none"> No short arm Correct training of the trunks, no too early grape harvest No short-pruned wounds (close to the trunks) to avoid drying zones inside the trunks Prefer the pruning systems that avoid constant changes of sap routes (Guyot-Poussard with stumps) Prophylactic methods (prune in late winter, namely for Eutypa) Protection of pruning wounds, preferably with a paste Avoid large pruning wounds (electric shears) Avoid excessive use of fertilizers

The epidemiology of Esca disease may begin very soon in the history of a young vine, notably from mother plants infected by *Phaeoconiella chlamydospora* (Fourie and Halleen, 2002). Different steps along the nursery process, like hydration and callusing, involve water soaking or high temperature (25-28°C), two environmental factors highly favourable to the fungal growth, among other factors well reviewed by Waite and Morton in 2007. However, the presence of wood fungi in a vine does not necessarily mean that this vine will become

diseased. This also depends on other predisposing factors during and after planting in the vineyard (Ferreira *et al.*, 1999; Stamp, 2001; Lecomte *et al.*, 2008). Therefore, in order to avoid a too early and rapid inner necrosis development in the wood of young grapevines, different preventive measures are now advised including recommendations applicable before, during and after planting (Table 3).

Conclusion

Many complex pathological scenarios could probably explain the grapevine trunk disease developments. But the high variability of situations, as far as its incidence is concerned, clearly indicates that Esca is a “consequence” disease resulting both from the influence of biotic factors and abiotic factors.

All the favourable cultural factors mentioned above, more likely when they are combined, may largely contribute and predispose some vines to unusual levels of disease expression or of microbial development into the wood.

In our opinion, control of trunk decline diseases will likely be based on the selection of tolerant cultivars and/or on managing the cultural factors than on the search of a hypothetic curative compound able to replace the sodium arsenite.

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