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New insights into Esca of grapevine: the development of foliar symptoms and their association with xylem discoloration

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ABSTRACT

A new study on the development of foliar symptoms of Esca was carried out from 2004 to 2006 in five mature vineyards in Aquitaine, France. Symptoms were monitored for severity and changes over time. Initial foliar symptoms were characterized by the presence of drying zones and/or discolorations (reddening or yellowing), which are symptoms that have also been attributed to Black Dead Arm

(BDA). Then, the less-severely affected leaves persisted throughout the summer and developed into typical 'tiger-stripe' symptoms of Esca. The most severely-symptomatic leaves fell soon after symptoms appeared. Severely-diseased vines showed typical apoplectic or acute forms of Esca that did not differ from the severe BDA forms. The appearance of canopy-symptomatic vines increased uniformly over time, reaching a maximum incidence by the end of July. A second survey in 41 European and Lebanese vineyards showed that longitudinal discolorations were visible under the bark of 95% of the vines showing foliar Esca symptoms. These wood symptoms, also previously attributed to BDA, appeared as xylem orange-brown stripes. Thus, foliar symptoms of Esca showed transitory phases which overlapped with some BDA descriptions. Most of these symptoms, in the west-palearctic regions that were investigated, were commonly associated with the presence of one or several xylem discolorations.

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Esca of grapevine, also known as 'black measles', is such a complex disorder that some authors have described it as a complex of fungal diseases (33,42). The aetiology of this syndrome is still a matter for study and many pathogenesis scenarios explaining the origin of foliar symptom expression are considered possible (35,42). A large diversity of foliar and wood symptoms has been associated with Esca and related declines, as reviewed in Table 1. No fungal parasite has been isolated from Esca leaf lesions (47). Foliar symptoms appear in late spring or summer and are traditionally divided into two forms depending on their severity and how rapidly they appear (1,11,14,33). The acute or apoplectic forms are characterized by a rapid wilting of the entire plant or of one arm or several shoots, as well as leaf drop, shrivelling and sometimes the drying of grape clusters (1,11,14,33). The "chronic" or "mild" form is associated with foliar symptoms described internationally as showing a tiger-stripe pattern (33). In black-berried cultivars this foliar symptom consists of characteristic multiple banding discolorations surrounding dry, brittle, light-brown or red-brown necrotic tissue on the leaf blade, which can appear as bordered by narrow red or yellow blotches. In white-berried cultivars the red color is normally absent. Leaf color alterations may start as small, pale or bright yellow spots between the primary veins gradually enlarging and sometimes becoming necrotic at the center during the course of the growing season (33). Interestingly, only Viala (47) stressed that necrotic zones may also originate from drying zones that at first appear pale green to greyish green. Moreover, varying degrees of red colorations occur on the leaves of red-berried cultivars (47), which may include dark red or wine-red symptoms, although these symptoms have also been attributed to the disease Black Dead Arm (9,39). Because, the wood of diseased vines is often severely decayed, the expression of foliar symptoms has been associated with the development of different fungi in the grapevine wood

(7,11,12,33,37). Cross sections of the trunk reveal a variety of lesions and/or decay types (20,29,32), which all impact the vascular system. It is generally agreed that these lesions will continue to expand as the plant ages, until the vine declines (25,33,42). In older vines, the wood usually shows a white to yellow soft rot, from which lignin-degrading basidiomycetes like *Fomitiporia* spp. (in Europe usually *F. mediterranea*) can be isolated (12). Esca lesions are also associated with different fungi including Phaeo's species (33, 42), but many other species considered either as saprobes or pathogenic could be also involved in the development of the esca syndrome (35).

Black Dead Arm (BDA) is another disease described as having slightly-different foliar symptoms from those associated with Esca (22). This wood disease was first described in 1974 by Lehoczky (28), who reported only the wood canker and no foliar symptoms (18,28). Moreover, another wood symptom was also later described as characteristic of BDA: the occurrence of an orange, then brown xylem discoloration appearing always associated with the foliar symptoms (22). Located in the outer xylem, this peculiar wood discoloration symptom appears as a superficial, longitudinal stripe just beneath the bark. The first link between cankers associated with Botryosphaeriaceae species and a reddening on the leaf blade of black cultivars was documented in 1978 and 1987 in Italy (9,39). Similarly, in France, foliar symptoms described as typical were reported from black-berried cultivars, beginning as drying zones and/or wine-red areas on the margins and on interveinal areas of the leaf blade (22).

Botryosphaeriaceae species have been isolated from these BDA wood lesions (9,22,39). More generally, members of Botryosphaeriaceae are presently considered as the causal agents of diverse trunk cankers of grapevine (2,6,17,31,36,43-45). In Australia, South Africa, and the US, these fungi have been recovered from wedge-shaped wood cankers, which are typical of the disease Botryosphaeria canker of grapevine (40,41,44,46). Parts of these sectorial cankers were originally thought to be due solely to *Eutypa lata*, the causal agent of Eutypa dieback, whereas the importance of the Botryosphaeriaceae as primary pathogens of grape was largely ignored (17). We now know that these species can also be isolated from all types of lesions encountered in vines that are simultaneously

affected by either Eutypa dieback or Esca (3,34). Canopy symptoms most often reported from vines with Botryosphaeriaceae-associated cankers are bud mortality, mild chlorosis, slow decline or reduced vegetative growth (4,30,36,43-44,46). Interestingly, these studies did not associate the Botryosphaeriaceae cankers with any foliar reddening during summer on black cultivars, such as those attributed to BDA (9,22,39). Thus, while the status of members of Botryosphaeriaceae as grapevine pathogens has been well documented, the relationship between the presence of these fungi within the grapevine wood and the occurrence of foliar symptoms of BDA, similar to those of Esca, is still not clear. This may be due to the fact that foliar symptoms of BDA have not been investigated thoroughly and that the mixed-fungal infections we commonly encounter in the field confound our ability as researchers to associate specific symptoms with individual diseases.

Some authors as well as preliminary observations in French vineyards indicated that Esca and BDA foliar symptoms may be related, because they are frequently observed on the same vine (13,23,27,42,43). For the same reason, field observers from the French National Wood Disease Survey considered it as a single foliar symptom category, "BDA-Esca" (13). In this context, we have revisited "BDA-Esca" symptoms, hypothesizing that symptom variability may result from a temporal change in symptom development. The two main objectives of our study were to show: i) the occurrence of a temporal development of foliar symptoms of Esca, and ii) the consistent association of a xylem stripe under the bark of vines with foliar symptoms of Esca. The first objective included the examination of a possible connection between foliar symptoms of Esca and those attributed to BDA. In order to achieve these objectives, it was necessary to carry out surveys relying on precise observations (i.e., within individual vines and within a vineyard) to acquire basic information on the development of such a complex symptomatology.

MATERIALS AND METHODS

Description and development of foliar symptoms in Aquitaine vineyards. In the Aquitaine region, five vineyards were studied from 2004 to 2006 (Table 2), to observe and assess the evolution of foliar

symptoms over the summer. These vineyards were selected due to their high potential for esca expression, rare Eutypa dieback incidence and low mortality due to other causes. Between 500 and 1,072 vines per vineyard were randomly sampled. All vineyards were mapped and the status of each vine was recorded using an adapted scale (Table 3) previously devised for Esca and Eutypa dieback symptoms (10). This scale corresponded to the presence/absence of foliar symptoms (leaf-symptomatic vines or leaf-asymptomatic, respectively), as well as trunk-affected vines (dead arm present or cut, restored vine, dead vine, re-planted vine,…). Foliar symptom severity was assessed based on involvement of one or both arms of the vine (Table 2) and levels of leaf damage from the less-severe symptoms (discolorations and spotting) to the most severe (large drying zones, wilting and defoliation), including the apoplectic form. Each vineyard was then characterized each year according to the overall % of trunk-affected vines, as well as the general incidence of vines with foliar symptoms whatever their severity level. Percentages were ranked following pair wise comparisons using a parametric z-test based on the binomial distribution (*P*-value threshold = 1.96, with $P = 0.05$ considered to be significant) [Statbox Pro version 5, Grimmersoft©, Issy-les- Moulineaux, France].

In 2004, at least six observations were recorded from mid-June (around mid-flowering) to the end of August or September (berry maturation stage). In 2005, vines were assessed on all sites from early June to the beginning of September at least once per week. In 2006, vineyards in Cénac and Latresne were investigated more thoroughly by surveying the sites twice per week in order to record foliar symptoms almost as soon as they have emerged. Foliar symptoms were recorded for each symptomatic vine as one of three categories as follows: 1) '**B',** BDA-like symptoms, leaves with typical interveinal reddening or the presence of drying zones, the latter of which may be surrounded by a reddening area specifically on red/black cultivars (Fig. 1 A-I); on white cultivars, drying zones that may be accompanied by interveinal chlorosis, as described by Larignon *et al.* (21); 2) '**E',** leaves with Esca-like symptoms, namely presence/absence of necrotic zones, various discolorations and a typical 'tiger-stripe' pattern (Fig. 1 M-P), and 3) '**BE**', both symptom patterns observed on the same vine simultaneously (Fig. 1 J-L).

Presence of vascular discoloration in European and Lebanese vineyards. From 2004 to 2007 a total of 41 vineyard-plots were monitored in different European and Lebanese regions, in addition to four of the five Aquitaine vineyards described above. The relationship between the presence of discoloration under the bark and the foliar symptoms (B and BE *vs* E) in symptomatic *vs* asymptomatic vines (Tables 2 and 4) was investigated. The sites were distributed in 34 different locations: 24 sites in France, eight in central Lebanon (Bekaa valley), one in northern Spain (Rioja Alavesa, Basque Country) and one in southwestern Germany (Tables 2 and 4). Agricultural practices were representative of each region. Vines were randomly selected among symptomatic plants that showed typical and marked foliar symptoms affecting a cane or several leaves. Foliar symptoms were assessed as described above.

Discolorations that appeared as longitudinal 'stripes' along the length of canes and/or trunks were revealed by detaching some pieces of the bark with a knife as soon as the foliar symptoms were seen in Aquitaine. For leaf-asymptomatic vines we completely peeled off the bark. The 34 vineyard sites surveyed provided a total of 688 grapevines: 581 leaf-symptomatic and 107 non-symptomatic. The 581 symptomatic vines were further divided into two subgroups: 104 vines with BDA-like symptoms (B and/or BE) and 477 vines with Esca-like symptoms (E). These distributions of vine counts were compared using both two-factor contingency tables (χ^2 test) and Fisher's exact test at $P = 0.05$.

During the survey, the location of the stripe(s) within the vine architecture and the sap routes was observed for 452 leaf-symptomatic vines. In particular, we looked for the presence of stripes either located close to and along old longitudinal cankers externally visible, or located in a line on either side of pruning wounds (sap route). In September 2007, a set of 29 vines showing typical Esca symptoms was examined for the presence stripes in the Latresne vineyard (Table 2). The location of each stripe was performed by slightly lifting the bark with a knife at two or three small spots (1 cm^2). Stripes were then labelled *in situ* with a drawing pin. In September 2008, the fate of the xylem stripes was checked for the possible development of a trunk canker.

RESULTS

Description and development of foliar symptoms in the Aquitaine region. The incidence of trunk and foliar symptoms varied greatly on the vines (2004-2006) (Table 5). As expected, the percentage of trunk-affected vines increased year after year and the differences between sites were significant. Vineyards located at Ludon-Médon, Cénac and Labarrère showed more than 30% incidence of trunk-affected vines, whereas the percentages were much lower in Latresne and Ramouzens. In 2006 (and 2005 as a trend), the highest percentage of vines expressing foliar symptoms (55.6%) and one of the lowest incidences of declining vines (9.6%) occurred in the Lyra-trained vineyard of Latresne.

Marked gradations of foliar symptom ratings were noticeable with regard to symptom severity and qualitative development either at the leaf level or at the vine level. Symptom expression was gradual and characterized by an increasing number of affected leaves on one or more shoots as well as on one or more arms, affecting the entire plant canopy at the final stage. Three main categories, namely **I**, **II** (including **IIA** and **IIB**) and **III**, explained below, could be defined according to the level of damage (extent of drying areas) and symptom evolution. In all sites, the first foliar symptoms to appear (Fig 1 A-I; Fig. 2 A, B, E, F, I and M) corresponded mostly to Black dead arm foliar symptoms as described previously (22). In black cultivars, they were characterized as interveinal drying and/or a wine-red pigmentation of the lamina, whereas in white cultivars, the pigmentation was yellow and appeared less frequently.

The primary symptoms were separated into the two main categories **I** and **II** according to their severity. Category **I** corresponded to very severely-affected leaves. These leaves generally exhibited very large drying zones and little or no pigmentation (Fig. 1 D). Most of them fell rapidly after several hours or days (defoliation). In the most severe cases, leaves were wilted (‘folletage’) indicating an apoplectic form (Fig. 3 J). Nevertheless, some leaves dried and remained attached to the canes (Fig. 2 P). Category **II** comprised foliar symptoms of intermediate or lower severity that remained attached to the canes. Two subgroups were then established depending on the development of the affected zones

and the occurrence of newly damaged zones. In **IIA**, leaves showed large or small interveinal drying zones with few changes in coloration during summer (Fig. 1 N and Fig.2 G, H compared to F). In **IIB**, less or midly damaged leaves generally showed wine-red (black cv.) or yellowish discolored zones (white cv.), often with limited or no drying zones as illustrated in Fig. 1 A-C, E-G, O and Fig. 2 A, E, I, M. Different color development and/or new discolorations could then appear in the lamina during the summer. On black cultivars, most leaves exhibiting such wine-red pigmentation at an early stage generally showed typical Esca tiger-striped patterns a few days or weeks later (Fig. 2). For white cultivars, the development mostly consisted of the emergence of a yellow discoloration around the drying zones and also elsewhere on the lamina (Fig. 1 O, P).

Category **III** consisted of various mild secondary, symptoms (mainly discolorations) that appeared either on symptomatic leaves as described earlier, or in their vicinity on the same or a neighbouring cane (Fig. 1 P and Fig. 2, K, L compared to J, O compared to N). These secondary symptoms developed mostly at the end of the season. Such symptoms could vary according to the cultivar and vineyard. They notably occurred in Lebanon on vines with several arms, where at least one arm was frequently affected by severe symptoms of wilting. Some of these symptoms were very similar to the color changes associated with natural leaf senescence that usually occurs in autumn. Finally, all the previously described categories (**I**, **IIA**, **IIB** and **III)** could be detected on the same cane. The severity of foliar symptoms could also significantly increase during the course of the season (Fig.2 L compared to I-K and P compared to M-O) and severely affected vines (acute forms) could also become completely apoplectic.

The results for the vineyard as a whole showed a general progressive pattern with a regular and sigmoidal increase in the number of vines exhibiting typical foliar symptoms throughout the summer (Fig. 4). In every situation (vineyard x year combination), vines exhibited on the same symptomatic part of the canopy the three profiles of foliar symptoms consecutively. The first typical foliar symptoms to appear corresponded mostly to BDA-like ones and were recorded as early as the beginning of June. In contrast, typical Esca-like symptoms were rare at first. Subsequently, the number

of vines showing BDA-like symptoms increased and generally reached a maximum by the end of July. During the same period and in parallel many vines showed different foliar discolorations or deteriorations that corresponded to both BDA-like and Esca foliar symptoms. The progression of such mixed symptoms reached a maximum between the end of July and the beginning of August. Finally, in late August to September, a great number of symptomatic vines showed a characteristic Esca tiger-striped pattern on the leaves that did not completely dry or fall during the summer.

Apoplectic forms on one or several arms were seen on all sites, but more frequently on two sites (Cénac and Ludon-Médoc) in 2005. The Cénac site best exemplified a number of apoplectic vines that gradually and slightly increased up to the end of the season. Their appearance was first associated with a severe form of BDA-like symptoms with either numerous wilted leaves or large interveinal wilting zones (grey to pale green). After several weeks, canes generally became completely dry, with brittle and pale brown necrosis, or they severely declined (Fig. 3 J). After some days or weeks, such vines were also indistinguishable from the typical apoplectic form classically ascribed to Esca. Depending on the vineyard x year interaction, the rate of development from BDA-like symptoms towards typical Esca symptoms, with either red bright or yellow colors, differed in speed. For example, in 2006, symptom progression was rather slow in Cénac and Latresne, where most vines still showed BDA-like symptoms at the end of June. However, in 2005, symptom evolution was more rapid in June showing a predominance of mixed or Esca symptoms in Ludon-Médoc and Latresne in less than three weeks.

Occurrence of xylem stripes in European and Lebanese vineyards. As previously described by Larignon *et al.* (2001), in all cases, longitudinal discolored stripes were located superficially in the outer xylem of the trunk and arms (Fig. 4). The stripes appeared concomitantly with foliar symptoms and in trunk xylem areas leading to canes bearing symptomatic leaves (Fig. 4 A, B, J). At an early development stage (1-3 days old), the altered wood appeared as a yellowish-orange stripe, then orange-brown, similar to the color of oxidized tissues (Fig. 3 D, E and H). In some cases, recent stripes appeared discontinuously and often extended along the trunk (Fig. 3 E-G, J and K), but not always

beyond the graft union. No precise initial points of developing necrosis could be identified in order to explain the origin of these stripes; neither a precise wound nor an altered zone that could possibly obstruct the vessels. In the most severe cases - namely apoplectic forms - several stripes (often 2 to 3) were observed which were often wide (2-5 cm) and deep (1-2 mm). It was generally observed that the number or the extent of lesions (depth, length or width) was associated with an increased severity of the foliar symptoms. Later in the season, several weeks after the vines had expressed foliar symptoms, the longitudinal stripes generally turned brown (Fig. 3 F, K), indicating the beginning of a perennial wood lesion.

The occurrence of a xylem discoloration under the bark from 2004 to 2007 was studied using 688 vines comprising 581 (84.5%) leaf-symptomatic vines and 107 (15.5%) asymptomatic ones (control). Most of the asymptomatic vines (98.1%) showed no longitudinal stripes. Among the symptomatic vines, only 4.3% lacked the stripe, showing a strong correlation between foliar symptom occurrence and the stripe (Fisher exact test, two tailed, $P<0.0001$; $\chi^2=519.1$; df=1; $P<0.001$). Symptomatic vines were used to test the hypothesis that the presence of the stripe was independent of the kind of foliar symptoms (B and BE *vs* E), including 104 vines assessed with BDA-like symptoms. Of these 104 vines 97.1% exhibited at least one xylem stripe in the wood confirming that these lesions are frequently related to this kind of foliar symptoms, as previously reported (21). The vines in which no stripe was seen (2.9%) showed rather mild BDA-like or Esca foliar symptoms associated with necroses already existing in the wood, in particular fresh necroses extending from pruning wounds. Similarly, among the other 477 vines that showed Esca-like symptoms, 95.4% also exhibited at least one longitudinal stripe. The proportions of symptomatic vines showing at least one stripe were not significantly different between vines showing either BDA-like or Esca symptoms (Fisher's exact test, two tailed, $P = 0.60$; $\chi^2=0.62$, df = 1; $P = 0.43$).

Three types of relationship between stripe location and vine architecture were identified in 452 vines. First, 47% of the vines exhibited at least one stripe which had formed in a sap route close to a large externally visible canker lesion zone (Fig.3 H) similar to that described by Branas (1974).

Second, 31% of the vines showed a stripe on a line from either side of pruning wounds (Fig. 3 G). Third, 22% of the vines showed a stripe which was associated with both locations. Overall 69% of the stripes were located close to a previous necrosis. One year after the first examination in 2007, 29 of the 34 vines examined in 2008 in the Latresne vineyard showed longitudinal necrotic lesions in the wood that corresponded exactly to the stripes labelled initially. 18 stripes were found to develop into longitudinal canker lesions one year later (Fig. 3 C and K) as described in other studies (5, 17). The others (11) were found to have merged with previously existing longitudinal canker lesions and were covered over by newly generated wood tissues.

DISCUSSION

The diagnosis of Esca *sensu lato* in grapevine during summer is complicated by the fact that symptom expression varies between black and white cultivars and with the location, and also because of many reports with different descriptions. Previous studies (13,23,27,30,42-43) suggested that BDA foliar summer symptoms could be related to those of Esca. Here, we had originally advanced the hypothesis that the early symptoms of drying zones and early reddening (black cv.) or yellowing (white cv.) previously attributed to BDA constitute a transient phase in symptom development within the Esca syndrome. This notion was supported by the fact that foliar symptoms were seen to develop during the course of the season and could be associated with wood stripes, a typical Esca symptom (1,8,14).

Regarding the leaves, our regular observations showed that those on Esca-diseased grapevines may host highly variable symptoms depending on their severity and age as largely suggested by literature. In contrast to most previous descriptions, Esca symptoms were not separated into mild or apoplectic forms, but according to a classification based on a gradual scale of severity, starting from some leaves showing only discolorations up to a complete vine wilting. At the early stage, in all sites, mild symptoms frequently corresponded to either a wine-red discoloration on black-berried cultivars or a yellowish discoloration on white-berried ones. More severe symptoms were generally characterized by the occurrence of interveinal drying zones (symptoms previously attributed to BDA). In most

severe cases, symptomatic leaves fell rapidly, corresponding to an apoplectic form. The first appearance of pale green-to-grey wilting zones before drying (42,47) was a frequent and original observation in our surveys. This observation is not in complete agreement with some initial descriptions of Esca (7,11), in which symptoms were described as starting from discolorations with the possible development of necrotic zones in their center. Such a sequence of symptoms, starting from a discoloration and developing in a drying zone, was also observed in our survey and corresponded to mild symptoms. However, such an evolution from discolorations to drying was much less frequent than the early symptoms described above. Following the appearance of the first symptoms, temporal changes in symptomatology on the leaves that did not fall included an increase in severity, with the extension of drying zones or discolored areas as previously reported (8,11,47). When there was no increase in severity, there was generally a change in color only: in black-berried cultivars, for example, a light red coloration often followed the initial wine-red coloration. During the course of the summer, further discoloration spots, corresponding to mild secondary symptoms, sometimes appeared on other leaves or on those previously altered. All these types of symptom development contributed to providing the typical Esca symptoms , *i.e*. a tiger-stripe pattern (11,32,42).

At the plot scale, the results for the foliar symptoms revealed a major progressive pattern in all the vineyards surveyed in Aquitaine: the symptoms increased up to a maximum more or less regularly in late July or early August. After this peak new symptomatic vines continued to appear up to September, although much less frequently. All symptomatic vines showed the development of BDA-like symptoms to Esca-like symptoms during the summer, confirming the results obtained for the leaves examined individually. The very high frequency of vines showing such a progressive development clearly favours a single syndrome, rather than two different and successive diseases always occurring in the same sequence. In contrast to conclusions drawn from previous data (1,11,42), disease development within the season clearly corresponded to a more or less regular process. This pattern was observed regardless of the site and the year, although 2005 was a dry year and the others more humid. This tends to indicate that the development of the disease may be dependent upon a

progressive phenomenon which would lead to this intensification of symptoms. Such a general pattern has already been described by Marchi *et al.* in 2006 (32). It should be highlighted that the first signs of Esca expression on a leaf appeared at the end of spring when mean temperatures progressively increased and water availability decreased. Further studies are therefore needed in order to better understand the links between environmental conditions (temperature, rain and water availability within the plant) and disease expression during the season, as well as the microbial activity in the grapevine wood.

In Aquitaine, significant differences in disease incidence between sites were noticeable. All monitored vineyards in the Bordeaux area exhibited a high percentage of leaf-symptomatic vines (> 15% in 8 cases out of 9 (Tab 5))., which was much higher than the mean incidence (less than 5%) reported in France (19). In this study, the percentage of trunk-affected vines was based, mostly, on visual observations of dead and missing vines or arms. This trunk-affected scale was indicative and relevant because all the Aquitaine vineyards surveyed were selected beforehand with a high incidence of esca and low mortality due to other causes (Eutypa dieback, Armillaria root rot, soil effect,…). Moreover, it has been demonstrated a positive and significant correlation between the incidence of dead vines (%) and the mean necrotic area (%) in the wood due to inner lesions typical of Esca (29). Thus, both variables, % of vines showing typical foliar symptoms and % of trunk-affected vines, are inseparable and perfectly illustrate the incidence of the disease. Leaf symptoms are generally the first signs of the decline. Trunk symptoms (externally visible) constitute the ultimate consequence of the esca disease. Incidentally, the only Lyra-trained vineyard (Latresne) showed the highest percentage of vines expressing foliar symptoms (55.6%), but one of the lowest incidences of trunk-affected vines. From this particular site, it can be hypothesized that there is an influence of the training system and the associated pruning practice on Esca incidence. Lyra-training system is based on the formation of two long arms (*ca*. 80 cm in length) bearing spurs distributed at every 20-30 cm. Arms of cordons often die more slowly that forms with very short arms like Guyot forms where an increased concentration of necroses is present in a limited wood zone (24-26). Cordons can thus express more often and longer

the trunk diseases foliar symptoms. Such an effect of a pruning method has been also reported in rootstock mother grapevines (29). Therefore, we would recommend that the relationship between the training system and foliar expression of Esca be further studied in order to identify and encourage the use of less-conducive training systems (11,16,24-26,38). We also recommend the use of the symptom scale developed in this study allowing observers to assess completely and precisely the incidence of Esca syndrome. A two-date survey, at 'veraison' and before harvest, would be sufficient to look for the evolution of foliar symptoms, including the transitory phase. The scale can be particularly useful for the selection of tolerant cultivars and/or determining the role of cultural factors.

In this study, we focused on an often neglected essential vine wood symptom, the occurrence of longitudinal xylem stripes, just under the bark that mostly develop into perennial wood lesions as already suggested (21). In the west-palearctic regions where our study was conducted, our results confirm that this wood alteration is a frequent and typical symptom of Esca decline, as reported by previous reports (1,8,14,23,27). The percentage of leaf symptomatic vines exhibiting such a stripe was very high (95%), irrespective of whether the leaf disorder was identified as BDA-like or Esca-like. All our observations in various vineyards in France and abroad, including some additional cases in Morocco and Italy (data not shown), showed at least one wood stripe on the same sap route that those of the cane bearing foliar symptoms. Our understanding in favor of a unique syndrome rather than two different diseases is substantiated by the fact that these stripes have been reported as a common symptom in both Esca and BDA. This view is also strongly supported by the fact that during the survey no difference was observed between the severe form of BDA (22) and the apoplectic form of Esca. Although there was no precise count, the number of stripes per vine and their size appeared visually to be connected with foliar symptom severity, such as wilting and drying zones on leaves. This was further substantiated by the fact that the few vines we examined not showing any stripes (4.3%) showed mild foliar symptoms only (mostly discolorations restricted to some leaves). Interestingly, this particular wood symptom has so far not been reported in the areas where apoplectic or severe forms of Esca are not seen [e.g., Chile (Auger, pers. com.)]. Therefore, further studies should

be carried out in other geographical locations, namely in climatic zones apparently less conducive to acute forms of Esca, to learn more about the presence of wood symptoms. Our observations of fresh stripes showed that the altered wood is sometimes discontinuous, with a yellowish-orange discoloration that is similar to oxidized tissue. The stripes were seen close to, but not strictly connected to, necrotic zones. Moreover, the origin of these longitudinal alterations could not be visually connected to a precise initial point of visible necrosis, neither a precise wound nor an altered zone that could possibly have occluded the vessels. Thus, the exact origin of the stripes remains unclear and two main hypothesises, not mutually exclusive, may be put forward. The stripes arise from: i) a fungal invasion, either from the outer bark or close to the inner necrosis, leading to vessel occlusions and/or to toxin diffusion, or ii) a sudden sap disruption, which is followed by foliar symptoms, as already suggested (23,27). An interesting point of aetiology in the esca syndrome is that there are non-revertible wood degradations (stripes and/or necrosis) associated with a revertible process of foliar symptoms (symptom appearance varies from year to year). It may be hypothesized that, each season, the newly-formed vessels, redefining the vascular system of the plant, may affect the foliar symptoms appearance (26). Other factors like plant defence efficiency or the pruning type that may interfer with the vascular system may also be of importance in foliar symptom development (16, 26).

Finally, from these two surveys, one in Aquitaine and another one in European and Lebanese regions, we conclude that the Esca syndrome encompasses a large diversity of foliar symptoms, due to severity or variations between red and white cultivars and a significant evolution during the course of the season. Furthermore, this syndrome is characterized by an original wood symptom, the xylem discolored stripe, appearing simultaneously with foliar symptoms and which could be responsible for their development. A better understanding of the origin of these stripes may be a key element for explaining the development of foliar symptoms and for making progress in the aetiology of this complex syndrome.

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1 **Table1**. Diversity of symptoms as reported in a selected literature review related to Esca or to Black Dead Arm descriptions

		Wood symptom		Foliar symptoms			
Disorder	**Reference**	**Internal**	**Stripe**	**Drying, dropping**	**Reddening**	**Yellowing**	**Chlorosis**
Esca	Viala, 1926 (47)	X[a]		X, green pale grey, brown	Red +/- bright	Variable	X
	Arnaud and Arnaud, 1931 (1)	X	X, light brown	X		X, veining	X
	Ciferri, 1955 (8)	X	X, 1-2 cm, brown	X, gradual	Variable		X
	Chiarappa, 1959 (7)	X		X	Dark red	X, clearing	X
	Geoffrion, 1971 (15)	X		X	X	X	X
	Branas, 1974 (5) Galet, 1995 (14)	X	X, brown	X		X	
	Mugnaï *et al.*, 1999 (33)	X		X	Tiger-striped pattern		X
	Dubos, 2002 (11)	X		X	X	X	X
	Larignon, 2004 (20)	X		X	Tiger-striped pattern		X

	Surico *et al.,* 2006 (42)	X	X	X	Tiger-striped pattern, variable		X
Black dead arm	Lehoczky, 1974 (28)		X, black canker				
	Cristinzio, 1978 (9)		X, dark	X	X		X
	Rovesti and Montermini, 1987 (39)	X[b]	X	X[c]	Wine red	X	
	Larignon *et al.,* 2001 (22)		X	X	Wine red, dark	None	
	Larignon and Dubos*,* 2001 (21)		X	X	Wine red	X, variable	X
	Auger *et al.*, 2004 (2)		X, canker	X	X		

[a] X: Symptom described in this reference. A blank indicates that the corresponding symptom has not been described in the reference

[b] Diffuse or limited cankers in the xylem

[c] Leaves can remain attached

Table 2. Main characteristics of the five Aquitaine vineyards (South-West of France) surveyed from 2004 to 2006 to examine the temporal evolution of Esca-like and BDA-like foliar symptoms and the occurrence of longitudinal discolored stripe(s).

Vineyard location[a]	Cultivar	Rootstock	Training system	Date of planting	Year(s) of observation	No of vines surveyed	No of vines peeled
Ludon-Médoc, Gironde, Médoc	'Cabernet Sauvignon'	5BB	Guyot, low form	1981	2004	1000	25
					2005	1000	72 + ***21***[b]
Cénac, Gironde, Entre-Deux-Mers	'Cabernet Franc'	3309C	Guyot, high form	1988	2004	500	6
					2005	500	4
					2006	550	2
Latresne, Gironde, Entre-Deux-Mers	'Cabernet Franc'	Fercal	Lyra, high form	1987	2004	1072	-
					2005	500	-
					2006	500	-
Ramouzens, Gers, Armagnac	'Colombard'	Gravesac	Guyot, high form	1990	2006	500	73
Labarrère, Gers, Armagnac	'Colombard'	SO4	Guyot, high form	1989	2006	500	83

[a] Municipality, Department, Appellation of the wine.

[b] In bold italic: number of leaf-asymptomatic vines examined as controls.

Table 3. Field rating used to assess the sanitary status (trunk symptoms) and the severity of foliar symptoms on the vines.

Observation of the whole vine	
Trunk symptoms	**Foliar symptom incidence[a]**
Arm entirely or partially dead (DA)[b]	Leaf-asymptomatic vine (0)
Missing arm (U)	Mild form on 1 arm, restricted defoliation, limited drying or discoloration (1)
= dead arm cut by pruning	Mild form on 2 arms, restricted defoliation, limited drying or discoloration (2)
Low growth, suffering vine (S)	Severe foliar symptoms on one arm, drying, defoliation (3)
Dead vine (M)	Mild foliar symptom on 1 arm and a severe one on the other(s) (1+3)
Absent (A)	Severe foliar symptoms on 2 arms and several canes, drying, defoliation (4)

Re-planted vine (CP)	Very severe foliar symptoms on 2 arms, acute defoliation (5)
Young vine (J)	Severe wilting on one arm, apoplectic form (Apo1)
Re-trained vine (R)	Severe wilting on two or more arms or on the entire vine (dieback) (Apo2)

1 [a] BDA-like and/or Esca-like (**B**, **E**, **BE**) foliar symptoms. Scale derived from those used by Lecomte and Darrieutort (2007).

2 [b] Between brackets, abbreviation or code used in this study

3

Table 4. Main characteristics of vineyards surveyed for the presence of sub-cortical and longitudinal discolored xylem stripes in European and Lebanese vineyards (2004 to 2007).

Vineyard location[a]	Climate[b]	Cultivar (no. of vines examined)[c]	Year
Castelnau d'Auzan, Aquitaine (F)	A	Ugni Blanc (1)	2004
Bonnetan, Aquitaine (F)	A	Cabernet Franc (3)	"
St Médard d'Eyrans, Aquitaine (F)	A	Cabernet Sauvignon (2)	"
St Julien-Beychevelle, Aquitaine (F)	A	Cabernet Sauvignon (1)	2005
Cahors, Aquitaine (F)	A	Merlot (1), Malbec (1)	"
Gaillac, Midi-Pyrénées (F)	A	Fer Servadou (3)	"
Eauze, Aquitaine (F)	A	Colombard (4), Baco (2 + ***1***[d])	"
Edde-Aana, West Bekaa (L)	M	Cinsault (4)	"
Skaff-Ammiq, West Bekaa (L)	M	Cinsault (3), Tfaifihi (1)	"
Ksara, Estephan, West Bekaa (L)	M	Cabernet Sauvignon (2)	"
Kanafar, West Bekaa (L)	M	Chardonnay (1), Merlot (1)	"
Kefraya, West Bekaa (L)	M	Cinsault (7)	"

Kouroum-Kefraya West Bekaa (L)	M	Syrah (6)	"
Mansoura, Central Bekaa (L)	M	Cinsault (1)	"
Tanail, Central Bekaa (L)	M	Cinsault (8), Ugni Blanc (4)	"
Gruissan, Languedoc-Roussillon (F)	M	Mourvèdre (23)	2006
Villeneuve les Maguelonne, Languedoc-Roussillon (F)	M	Danuta (15)	"
Castries, Languedoc-Roussillon (F)	M	Cabernet Sauvignon (26)	"
Fronsac, Aquitaine (F)	A	Cabernet Franc (20 + ***21***)	"
Villenave d'Ornon, Aquitaine (F)	A	Cabernet Franc (1), Cabernet Sauvignon (8), Chasselas (2), Chenin (3), Clairette (1), Gamay (1), Gewurztraminer (3), Pinot gris (1), Pinot noir (1), Riesling (1), Syrah (2)	"
Elvillar, Rioja Alavesa (S)	M	Tempranillo (26 + ***15***)	"
Avize, Champagne (F)	C	Chardonnay (7)	2007
Avize, Champagne (F)	C	Pinot noir (7)	"
Colmar, Alsace (F)	C	Gewurztraminer (7)	"

Ammerschwihr, Alsace (F)	C	Riesling (6)	"
Beaune, Burgundy (F)	C	Pinot noir (21)	"
Jonzac, Poitou-Charentes (F)	A	Ugni Blanc (24)	"
Ludon-Médoc, Aquitaine (F)	A	Cabernet Franc (26 + ***26***), Cabernet Sauvignon (25 + ***23***)	"
Freiburg-im-Breisgau, Baden-Württemberg (G)	C	Muller-Turgau (1), unknown (2)	"

[a] Municipality, Region, Country code: France (F), Lebanon (L), Spain (S) and Germany (G)

[b] Climate code: atlantic (A), continental (C), mediterranean (M).

[c] Vines either asymptomatic or showing BDA-like or Esca-like foliar symptoms during the growing season.

[d] In bold italic: number of asymptomatic vines examined as controls.

Table 5. Disease incidence in the five vineyards surveyed in Aquitaine region: % of vines with trunk symptoms and number and % of vines showing leaf damage, with either BDA-like or Esca-like foliar symptoms, during the growing season. Means followed by the same letter are not significantly different.

Municipality	Year(s) of observation	% trunk-affected vines	Number and (%) of vines showing leaf damage
Ludon-Médoc	2004	45.2 b	223 (22,3) e
	2005	49.8 a	72 (7,2) g
Cénac	2004	31.4 d	93 (18,6) ef
	2005	38.8 c	84 (16,8) f
	2006	48.2 ab	141 (25,6) de
Latresne	2004	Not recorded	487 (45,4) b
	2005	9.4 e	175 (35) cd
	2006	9.6 e	278 (55,6) a
Ramouzens	2006	8.7[z]	198 (39,6) c

Labarrère	2006	33.4 cd	151 (30,2) d

[z] In Ramouzens, the % affected vines was biased because only the dead vines were surveyed.

Figure Captions.

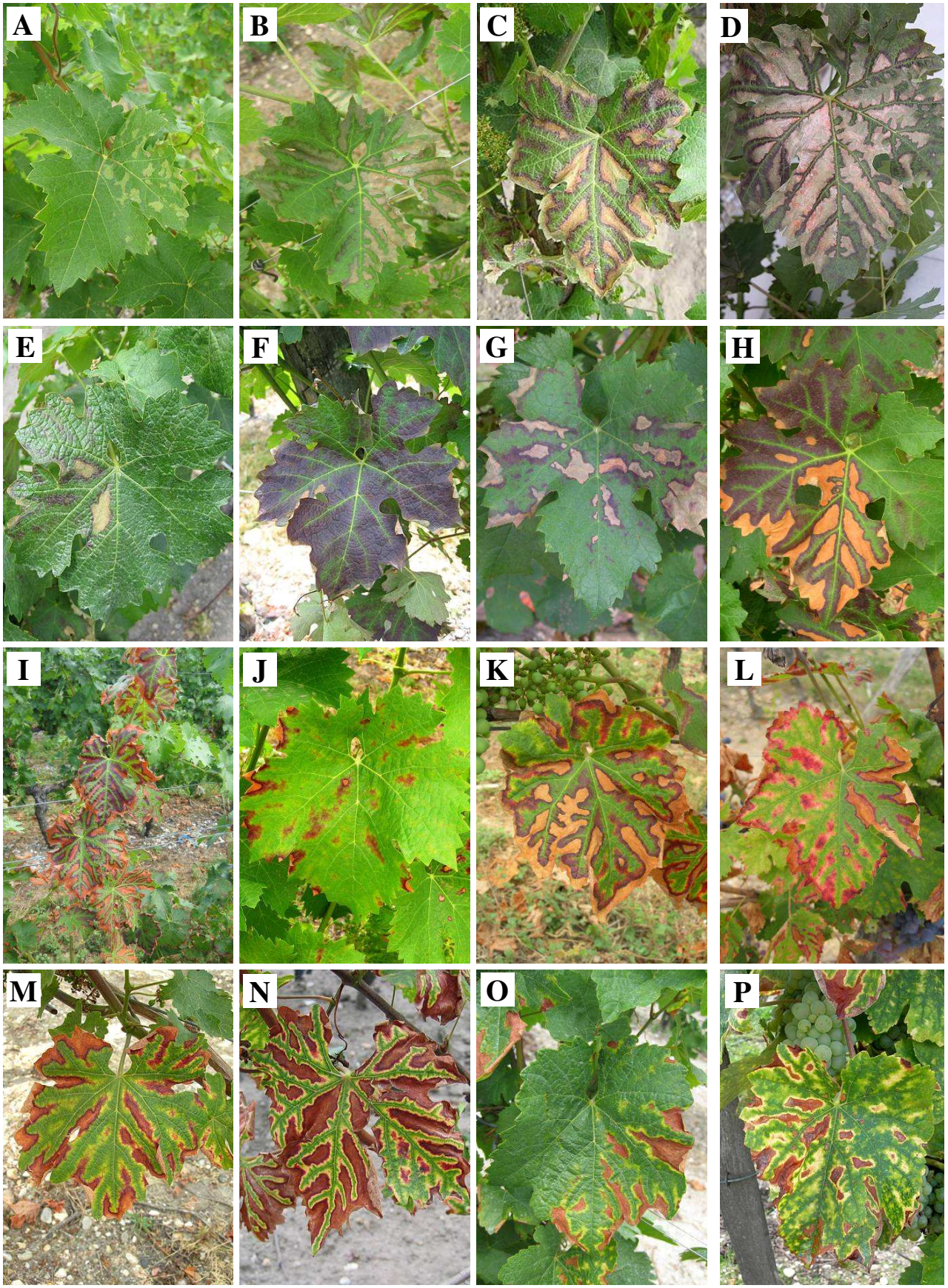
Fig. 1. Typical foliar symptoms taken into account beforehand for the disease-like classification (**B**, **BE**, **E**), which varied depending on cultivar (black or white), symptom age and severity (with the exception of wilted leaves). A-I: early symptoms that were classified as BDA-like symptoms, with no presence of yellow tissue (**B**). J-L: leaves that simultaneously presented BDA-like and Esca-like symptoms (**BE**). M-P: typical Esca symptoms (**E**) including the tiger-striped pattern with a yellow band between the green and red/necrotic tissue (M-N). A-N: black cultivars. O-P: white cultivars. A-E, G: symptoms less than 3-4 days old. F, H-P: symptoms several days or a few weeks old. Severe symptoms generally showed large drying zones on the lamina as shown by picture D (Category **I**); afterwards, this kind of leaf generally falls rapidly. Intermediate symptoms (Category **IIA**) concern leaves showing either large or small interveinal drying zones but with few changes in coloration during summer as shown by picture N. Mild symptoms (Category **IIB**) include limited drying zones, reddening (black cv.), discolorations and color changes depending on symptom age (A-C, E-M, O-P). Late or secondary symptoms (Category **III**) mostly consist of limited discolored spots that appeared on the lamina after the drying zones, as shown on picture P, compared to picture O.

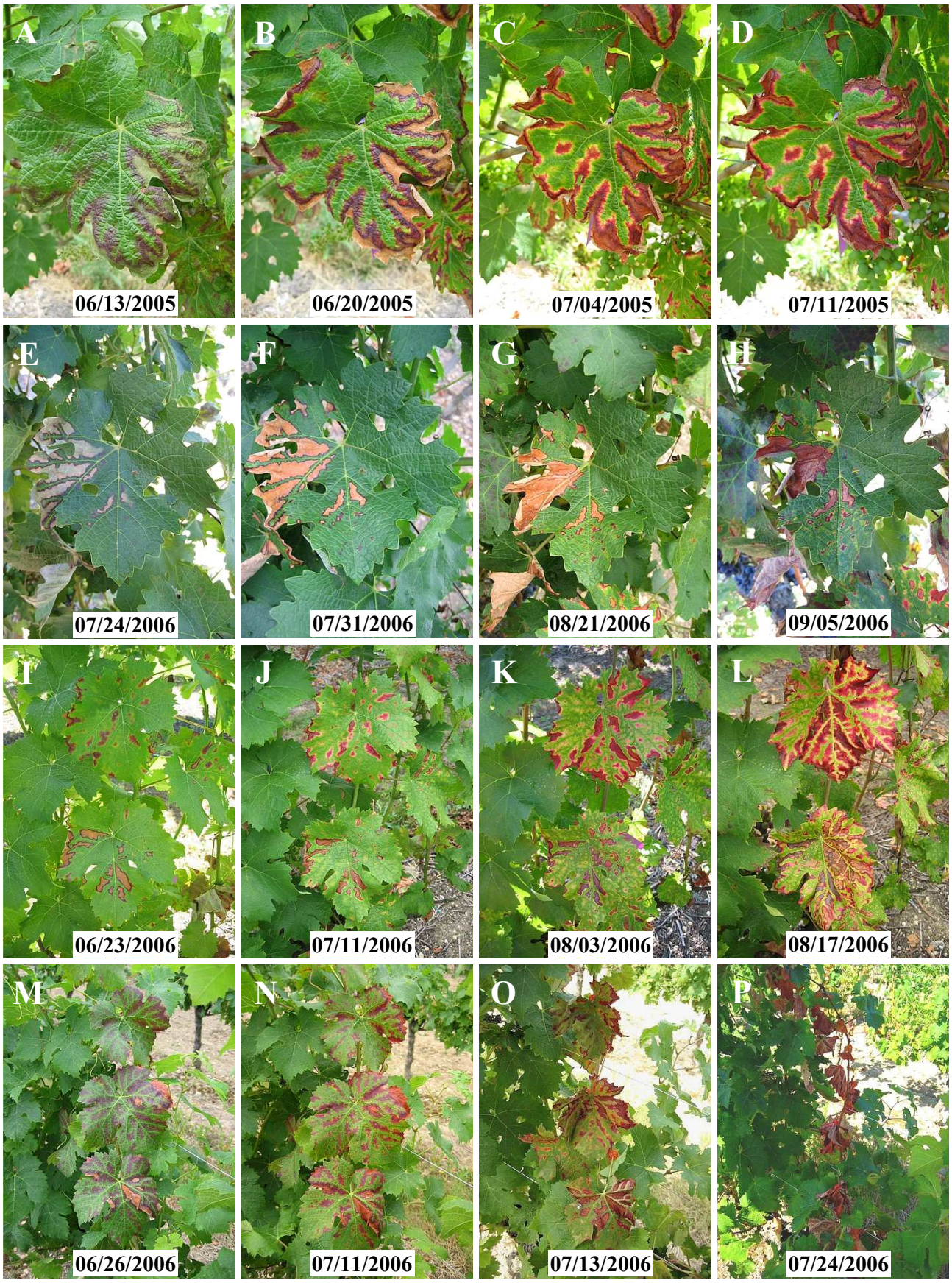
Fig. 2. Four examples of the evolution of mild symptoms on leaves affected by Esca on a black cultivar, Cabernet sauvignon, recorded in summer 2005 and 2006 in the Aquitaine Region. On the left, recent grey dry zones (A-B) or older brown dried zones (I, M) surrounded by red-wine coloration ; in the middle, the grey zones became completely dry and orange-brown (B, F); on the right, appearance of bright red or yellow coloration (C-D, H), often instead of the red wine pigmentation (C-D compared

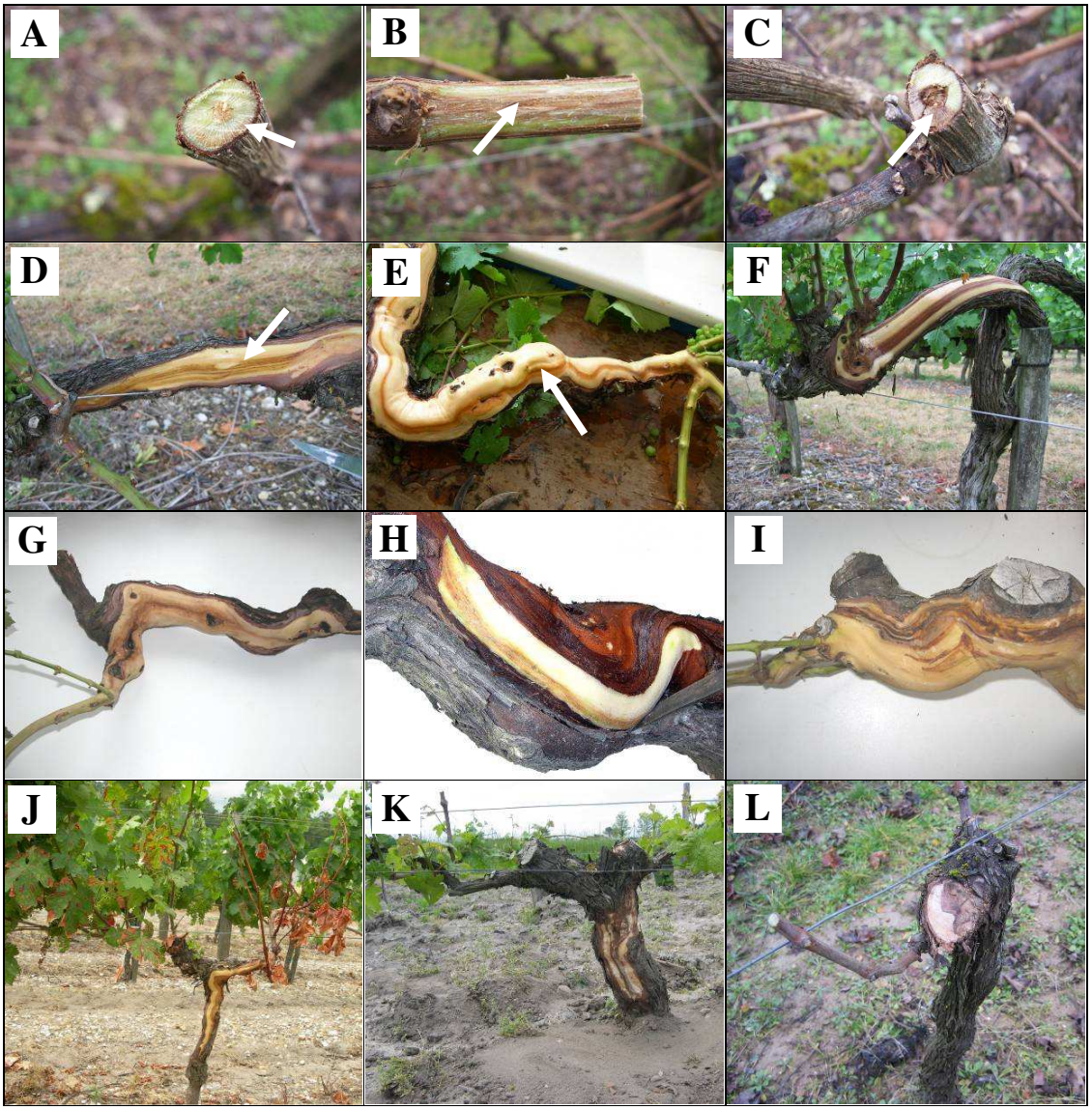
to A-B, J compared to I, N compared to M) leading to a typical tiger striped pattern of Esca (D, L, O). In the two cases, I-L and M-P, the severity of symptoms significantly increased as leaves aged.

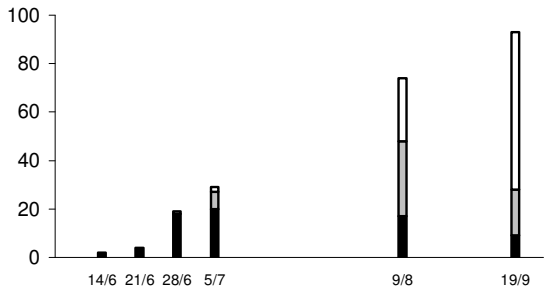
Fig. 3. Examples of wood symptoms associated with Esca on either canes or trunks. A: fresh orangey stripe lesion (white arrow) in the outer xylem under the bark of a transversally cut-cane; B: similar lesion observed after the removal of the bark; C: evolution after some months of a longitudinal lesion in a wedge-shaped canker; D: superficial and discontinuous lesion recently formed; E: similar lesion that appeared along a cane down to the bottom of a trunk; F: other lesion that became more brown some weeks after the foliar symptom appearance; G: longitudinal stripe that was seen in places under the bark resulting from pruning wounds; H, I: longitudinal stripes that occurred along dead wood zones; J: longitudinal stripe in relationship to leaf wilting and a severe form on one arm; K: old longitudinal stripe that formed a canker lesion; L : V-shaped canker that may often originate from a longitudinal lesion.

Fig. 4. Evolution of foliar symptom observed in five vineyards in summer in the Aquitaine region (South-West of France). Each date represents the incidence of vines showing either i) BDA-like symptoms (dark), ii) Esca-like symptoms (white), or iii) simultaneous symptoms corresponding to both symptomatologies (grey) or iv) apoplectic form (scratched area).









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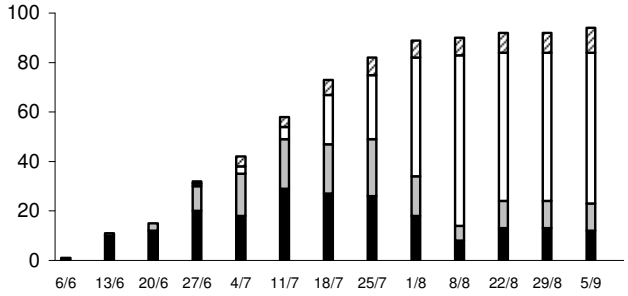
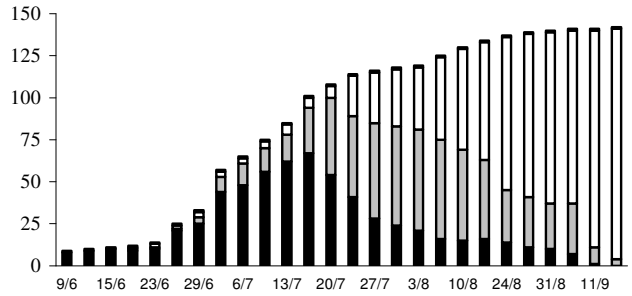
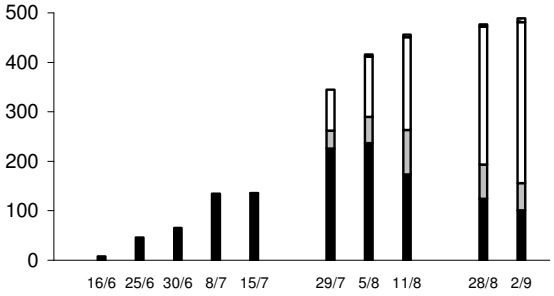


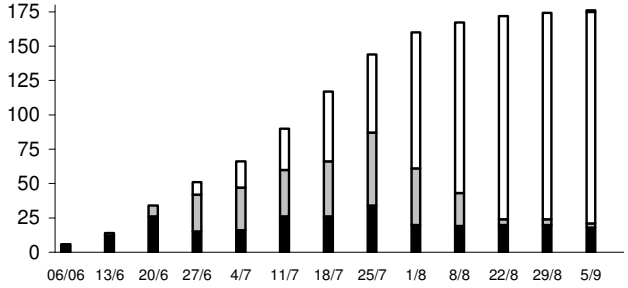
Cénac - 2005



Cénac - 2006



Latresne - 2004



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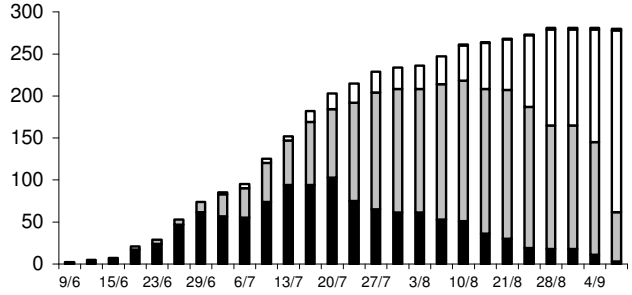
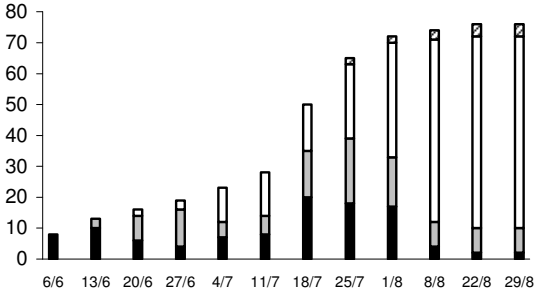
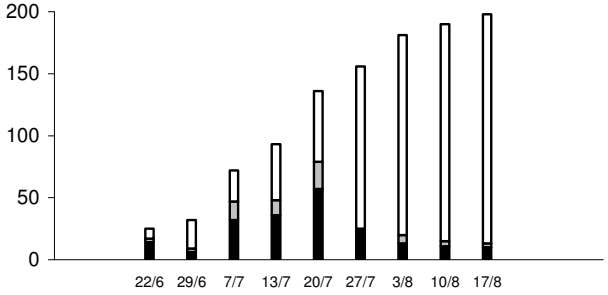


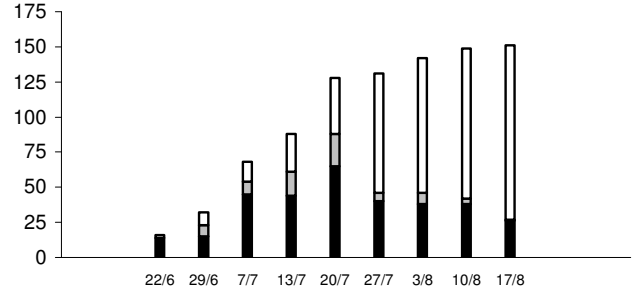
Latresne - 2006



Ludon Médoc - 2005



Ramouzens - 2006



Labarrère - 2006