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Esca, BDA and Eutypiosis: foliar symptoms, trunk lesions and fungi observed in diseased vinestocks in two vineyards in Alsace

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Summary

The French vineyard is affected by three principal wood diseases: Eutypa dieback, esca and black dead arm (BDA). *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum*, *Eutypa lata*, *Fomitiporia mediterranea*, *Diplodia seriata*, *Diplodia mutila* and *Neofusicoccum parvum* are the main fungi isolated in France and associated with grapevine trunk diseases. The aim of this study was to highlight the type of wood lesions and the fungus present in the Alsace vineyards (France) and to compare it with those identified in the other french vine-growing regions or with the German vineyards. Therefore, we have studied two vineyards with two different grapevine varieties ('Auxerrois', 'Gewürztraminer'). The foliar symptoms showed that the plots planted with 'Auxerrois' and 'Gewürztraminer' varieties had respectively 12 and 21 % grapevines with symptoms in 2005. Different cross sections were made on trunks and arms of 55 vines showing foliar symptoms, totalizing 162 microbiological isolations. Visual characterisations of the different lesions were described. The isolations made from the different necrosis showed the presence of species of fungi involved in grapevine trunk diseases and other fungi. Microbiological observations showed that for the Auxerrois vineyard the majority of the vines were infected with *D. seriata*, *P. chlamydospora*, *E. lata* and *F. mediterranea*. In the Gewürztraminer vineyard, the fungus most frequently isolated was *P. chlamydospora*, followed by *D. seriata*. The presence of *D. seriata* in different parts of the grapevine wood and in young wood is related to the severe damages observed on the vegetation.

Key words: esca, black dead arm, wood lesions, vineyard.

Introduction

Beside the fungi damaging the annual parts of the grapevine *Vitis vinifera*, those attacking the perennial parts of the vinestock are of great concern too. These fungi are supposed to cause the diseases called grapevine trunk diseases. These diseases, by generating an untimely death of

the vinestock, can lead to a renewal of the stocks which can reach more than 10 % of a vineyard. The incidence of the diseases increased over the last 10-15 years (CHIRAPPA 2000, GRANITI *et al.* 2000, GROSMAN 2005). Until the late nineties, beside eutypa dieback, the most common trunk disease in France was esca (LARIGNON *et al.* 2000 a). But at the turn to the new century, a third and new disease for France, called black dead arm (BDA), was identified in Bordeaux (LARIGNON *et al.* 2000 b, LARIGNON and DUBOS 2001) and afterwards in Champagne (Panon 2000) and in other French vine-growing areas (LARIGNON *et al.* 2001). A large scale survey initiated in 2003 for these diseases shows the importance of esca and BDA in most of the French viticultural areas, and the constant or quite constant increase of its incidences, but never decrease (GROSMAN 2005, FOTRE MULLER 2006). *Phaeoconiella chlamydospora* (W. Gams, P.W. Crous, M.J. Wingfield and L. Mugnai) Crous & W. Gams (as *Phaeoacremonium chlamydosporum*), *Phaeoacremonium aleophilum* (W. Gams, P.W. Crous, M.J. Wingfield and L. Mugnai), *Eutypa lata* (Pers.:Fr) Tul. & C. Tul. and *Fomitiporia mediterranea* (M. Fischer) (as *Phellinus punctatus*) were the main fungi isolated in vine stocks showing esca disease (LARIGNON and DUBOS, 1997). *Diplodia seriata* De Not. (as *Botryosphaeria obtusa* (Schwein.) Shoemaker), *Diplodia mutila* Fr. (as *Botryosphaeria stevensii* Shoemaker), and *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. were the principal fungi isolated in Bordeaux in vine stocks showing BDA disease (LARIGNON and DUBOS 2001). The *B. dothidea* isolates collected in France by Larignon and Dubos (LARIGNON and DUBOS, 2001), were later identified as *Neofusicoccum parvum* (LAVEAU *et al.* 2009).

P. chlamydospora and *P. aleophilum* are associated with Petri and esca disease (GRANITI *et al.* 2000, ESKALEN and GUBLER 2001). *E. lata* is responsible for eutypa dieback (MOLLER and KASIMATIS 1978). *F. mediterranea* could be considered as a secondary invader in France and is the main pathogen causing white rot in esca-infected diseased grapevines in Europe (LARIGNON and DUBOS 1997). *Botryosphaeria* species are latent pathogens of plants (SLIPPERS *et al.* 2007), responsible in viticulture for different symptoms such as BDA (LEHOCZKY 1974, CRISTINZIO 1978, PHILLIPS 2002, AUGER *et al.* 2004, VAN NIEKERK *et al.* 2006, SAVOCCHIA *et al.* 2007, REGO *et al.* 2008).

Field observations have shown that BDA produces an interveinal orange-yellowish pigmentation of the leaves in white grape varieties, and an interveinal red pigmentation of the leaves in red grape varieties, the symptomatic tissues becoming finally necrotic. For white grape varieties, the pigmentation in case of BDA has sometimes the appearance of downy mildew oil-spots-like discolourations. For the red grape variety 'Cabernet-Sauvignon', the differences in the foliar symptoms between BDA and esca, have been shown to be related in analytical differences of anthocyanidic compounds (LARIGNON *et al.*, 2003). The differences in foliar symptoms observed between BDA and esca for the white grape variety 'Sauvignon', are linked to analytical differences in some pigments, too (DARNÉ and LARIGNON unpublished data). Brown streaking of the wood under the bark is also observed (LARIGNON and DUBOS 2001).

The aim of this study was to highlight the type of wood lesions and the fungi present in Alsace vineyards (France), and to ascertain the presence of BDA, since this disease is known to be present in the Champagne at quite 300 km away from Alsace (PANON 2000), but not recorded in Germany, for example in the close vineyards of Baden Württemberg, only distant from Alsace vineyard about 30 km (FISCHER and KASSEMAYER 2003). In Germany, the dieback of the vines is related to esca disease, despite that the presence of some *Botryosphaeriaceae* is shown by microbiological isolations (FISCHER and KASSEMAYER 2003).

Material and Methods

Characterization of the vineyard: The two experimental plots were located in the Alsace vineyard in the east of France. The first experimental plot was planted in 1987 with *Vitis vinifera* 'Gewürztraminer' grafted on 161-49 C and SO4 rootstocks with a total of 1946 vinestocks. The second experimental plot was planted in 1976 with *Vitis vinifera* 'Auxerrois' grafted on SO4 rootstock. This plot counts 1050 vinestocks. The vines were trained in "double Guyot". These vineyards were never sprayed with sodium arsenite.

Observation of symptoms on herbaceous parts: The symptoms of grapevine trunk diseases were checked vine per vine during the vegetative growing period from 2004 to 2007. We recorded symptoms of eutypa dieback, esca and BDA, from the stage of "flower buttons separated" to the harvest of the grapes. We noticed the kind of symptoms, if chronic, what means a slow declining of the vegetation, or if apoplectic, what means a sudden wilting of the vegetation. The results were expressed as an incidence of each decline for the vineyard.

Trunk lesions and microbiological diagnosis: This point was conducted only in 2004 and 2005, and not for the whole period of observation of symptoms on herbaceous parts. There was no need to maintain the microbiological diagnosis for the subsequent years, because the kind of herbaceous symptoms did not differ from one year to another.

55 plants showing foliar symptoms in 2004 and 2005 were sectionned into 3-4 parts and investigated for both vineyards. The repartition between plots and years is given in Tab. 1. We noticed the kind of lesion observed in the wood, from the trunk to the one-year-old cane. 162 microbiological isolations were made from different zones at the border of the necrotic tissues. For each lesion investigated we cut 5 small chips (1 mm³), surface sterilised by submersion in a bleach solution (3 % active chlorine) for 4-5 s and placed on 9 cm diameter Petri dishes containing MEA (Malt Extract Agar: Cristomalt 15 g·L⁻¹ and Agar 20 g·L⁻¹). The dishes were incubated at laboratory conditions (temperature ranging from 20 to 25 °C; night/day alternance) for 4 to 5 weeks, until the fungal colonies could be identified to determine the fungi.

Table 1

Repartition of the plants for the study of trunk lesions and microbiological diagnosis

Variety	2004	2005
Gewurztraminer	11	10
Auxerrois	15	19

Environmental monitoring: Temperature and rainfall were recorded daily by Météo-France for the Gewürztraminer vineyard (Domaine du Hohrain N°68287003, alt. 285 m, lat. 47°57'9N, long. 07°17'2E) and by Alsace Appro (Turckheim, alt. 233 m, lat. 48°04'1N, long. 07°17'1E) for the Auxerrois vineyard.

Results and Discussion

Observation of symptoms on herbaceous parts: The observation of the expressed foliar symptoms in 2004 and 2005 showed that the plot planted with the 'Auxerrois' variety presented respectively 15 and 12 % of grapevines with trunk disease symptoms. In 2004 and 2005 respectively 35 and 50 % of these vines showing foliar symptoms, showed only BDA symptoms, as at the same time those showing only esca represented less than 10 %. The vines showing eutypa dieback reached around 15 % for both years. Thus, for respectively 40 % and 25 % of the vines showing symptoms, the symptoms consisted both in esca and BDA, if chronic or apoplectic.

The plot planted with the Gewurztraminer variety presented respectively 18 % and 21 % of the vines with trunk disease symptoms. In this plot the vines showing both BDA and esca symptoms were 50 % of the symptomatic vines in 2004, far less than in 2005 where they reached 80 %, mostly the chronic form for both years. BDA alone was encountered in quite 45 % of the diseased vines in 2004, but only in a good 10 % of the symptomatic vines in 2005. There were very few grapevines showing only esca symptoms. Eutypiose symptoms were not observed in this vineyard.

All along the study, it was not possible to correlate the development of the foliar symptoms with any climatic

factor such as temperature or rainfall (data not shown). As shown for example in Fig. 1, it was just possible to notice that the symptoms of BDA usually appeared at the time of flowering or just before it, far earlier than those of esca, which appeared during the herbaceous growing stage of the fruits, mostly around bunch closure, increasing rapidly its incidence. We noticed no shift of the foliar symptoms from BDA to esca: what appeared as BDA early in the season, remained as BDA all along the season, but could nevertheless be accompanied by symptoms of esca appearing later in the season. We care to precise that the expression of foliar symptoms remained on the same incidence levels

in the following years: respectively 18, 21 and 20 % for the plot planted with 'Gewürztraminer' and 14, 15 and 17 for the other one, in 2006, 2007 and 2008.

Trunk lesions and microbiological diagnosis: Visual characterisations of the different lesions are based on the necrotic types established by LARIGNON and DUBOS (1997) and LARIGNON and DUBOS (2001). In our case, for the trunks of the two grapevine varieties, we could find different aspects of necrosis: 1) A central area with light coloured necrosis and soft texture (Fig. 2 A, a) surrounded by a central brown area of hard consistence (Fig. 2 A, b) and a brown stripe in the wood af-

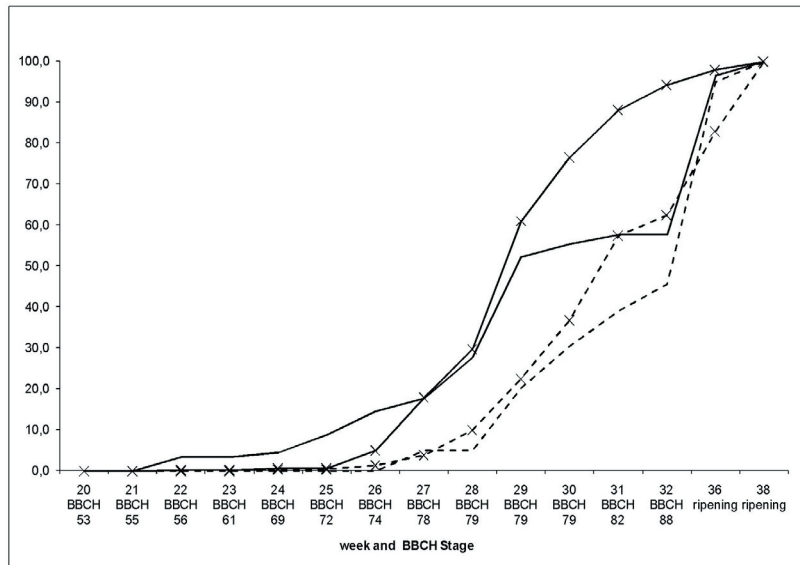


Fig. 1: Cumulative percentage of symptomatic vines on herbaceous parts for the 'Gewürztraminer' in 2005 (Black continuous line: BDA apoplectic; black continuous and marked line: BDA chronic; black dotted line: esca apoplectic; black dotted and marked line: esca chronic).

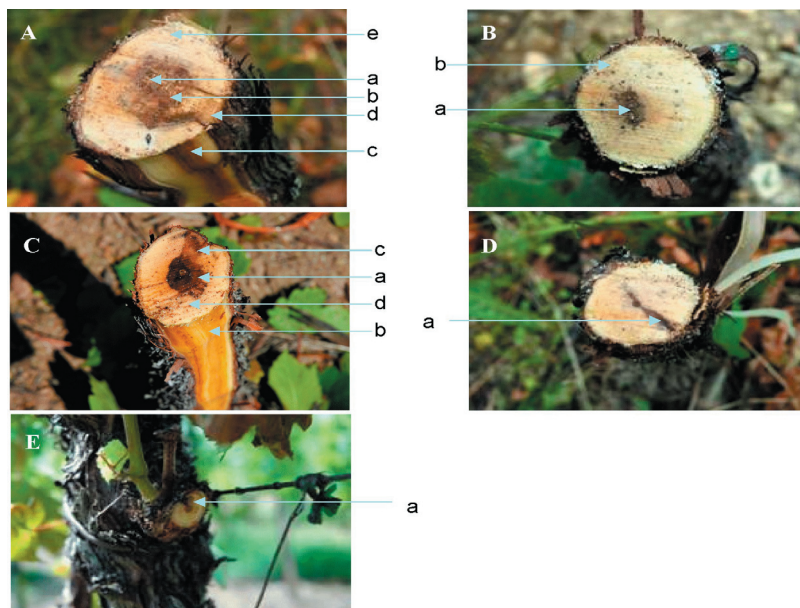


Fig. 2: Cross sections of trunks and arms with typical lesions symptoms. **A:** Association between central (a) and sectorial (d) soft necrosis, central hard necrosis (b) with a brown stripe (c) and punctuations (e). **B:** Central hard necrosis (a) and punctuations (b). **C:** Association between central (a) and sectorial (c) hard necrosis, with brown stripe (b) and punctuations (d). **D:** Fan shaped hard necrosis (a). **E:** Brown to grey-brown necrotic areas (a) and orange punctuations in one to six-year-old wood.

ter removal of the bark (Fig. 2 A, c) and a sectorial necrosis of soft texture (Fig. 2 A, d), different punctuations could be found in the healthy wood (Fig. 2 A, e); 2) A brown central area with coloured wood of hard consistence (Fig. 2 B, a) with punctuations in the healthy wood (Fig. 2 B, b); 3) A brown central area with hard consistence (Fig. 2 C, a), a brown stripe in the wood after removal of the bark (Fig. 2 C, b) and a sectorial necrosis of hard consistence (Fig. 2 C, c), different punctuations could be found in the healthy wood (Fig. 2 C, d); 4) A brown central area with sectorial brown fan shaped necrosis (Fig. 2 D, a). In addition, in one to six-year-old wood of the arms we found: 5) Brown to grey-brown necrotic areas and orange punctuations (Figure 2 E, a).

As shown in Tabs 2 and 3, the isolations made from the different lesions showed the presence of different species of fungi involved in grapevine trunk diseases, among other species, *Cladosporium* sp., *Penicillium* sp., *Epicoccum* sp. and *Pochonia bulbillosa*. The different fungi were present alone or in different combinations (Tab. 2).

In 'Auxerrois', microbiological isolations revealed that the majority of the investigated vines were infected with *D. seriata*, *P. chlamydospora*, *E. lata* and *F. mediterranea*. In the 'Auxerrois' vineyard, the lesions observed in the wood and the microbiological isolations confirmed the presence of BDA and esca detected through vegetation symptoms observations. Eutypa dieback sectorial necrotic areas were also almost systematically found in the wood.

In the Gewürztraminer vineyard, the most frequently isolated fungus was *P. chlamydospora*, followed by *D. seriata*.

The examination of 55 diseased plants through 162 microbiological isolations in diseased parts of trunks and arms enables us to ascertain the presence of various fungi involved in grapevine trunk diseases (Tab. 2), and to correlate this to the foliar symptoms of both esca and BDA observed for the investigated plots. For these correlation between symptoms in the wood and on the leaves, we can not separate esca from BDA, because as explained above, in most of the cases the vinestocks showed both esca and BDA symptoms on the leaves.

Generally we found *P. chlamydospora* in the central brown area of hard consistence (Tab. 3), in the punctuations in the healthy wood or in the sectorial brown fan-shaped necrosis. *F. mediterranea* occurred mainly in the necrosis of soft texture, if central or sectorial (white rot). *D. seriata* was often encountered in brown stripes with *E. lata* and in sectorial necrosis of hard consistence, where *E. lata* was the main fungus isolated. We detected also *D. seriata* with a high frequency in young wood (one to six-year-old) at the top of the trunk or between the first nodes of shoots, with typically sectorial grey-brown necrotic areas or brown-orange punctuations. *P. aleophilum* was poorly isolated, only in the central brown area of hard consistence.

The detection of *P. chlamydospora* in soft necroses or in sectorial necrosis of hard consistence, or of *F. mediterranea* and *E. lata* in central brown necrosis of hard consistence, may possibly be due to the superimposition and close vicinity of the different necrosis, and the advanced stage of

Table 2

Identity of fungi or combinations of fungi isolated from the different necrotic areas from cross section of trunks and arms with typical lesions

Central area with light-coloured necrosis and soft texture (Fig. 2 A: a)	<i>F. mediterranea</i> + <i>Cladosporium</i> sp. or <i>F. mediterranea</i> + <i>Epicoccum</i> sp. + <i>Penicillium</i> sp. or <i>F. mediterranea</i> + <i>E. lata</i> or <i>P. chlamydospora</i> or <i>S. hirsutum</i> or <i>D. seriata</i>
Brown stripe (Fig. 2 A: c; Fig. 2 C: b)	<i>D. seriata</i> or <i>E. lata</i> or <i>P. chlamydospora</i>
Sectorial necrosis of soft texture (Fig. 2 A: d)	<i>F. mediterranea</i>
Punctuations in the healthy wood (Fig. 2 A: e; Fig. 2 B: b; Fig. 2 C: d)	<i>P. chlamydospora</i> or <i>P. chlamydospora</i> + <i>D. seriata</i> + <i>Epicoccum</i> sp. or <i>Pochonia bulbillosa</i> or <i>E. lata</i>
Central brown area with hard consistence (Fig. 2 A: b; Figure 2 B: a; Fig. 2 C: a)	<i>P. chlamydospora</i> or <i>E. lata</i> or <i>F. mediterranea</i> or <i>F. mediterranea</i> + <i>Epicoccum</i> sp. + <i>Penicillium</i> sp. or <i>D. seriata</i> or <i>P. chlamydospora</i> + <i>E. lata</i> or <i>P. chlamydospora</i> + <i>F. mediterranea</i> or <i>Phomopsis viticola</i> or <i>P. aleophilum</i>
Sectorial necrosis of hard consistence (Fig. 2 C: c)	<i>E. lata</i> or <i>D. seriata</i> or <i>P. chlamydospora</i> or <i>F. mediterranea</i> + <i>Epicoccum</i> sp. + <i>Penicillium</i> sp. or <i>P. viticola</i> or <i>Phomopsis</i> sp.
Sectorial brown fan-shaped necrosis (Fig. 2 D: a)	<i>P. chlamydospora</i> or <i>E. lata</i>
Brown to grey-brown necrotic areas and brown-orange punctuations (Fig. 2 E: a)	<i>D. seriata</i> or <i>P. chlamydospora</i> or <i>Stereum hirsutum</i>

Table 3

Fungal organisms in esca, BDA and eutypa-affected grapevines

Necrosis	Fungal organism	Frequency (%)
Central area with light-coloured necrosis and soft texture and sectorial necrosis of soft texture	<i>F. mediterranea</i>	50
	<i>P. chlamydospora</i>	50
	<i>E. lata</i>	13
	<i>D. seriata</i>	6
	<i>S. hirsutum</i>	6
Central brown area with hard consistence	<i>P. chlamydospora</i>	67
	<i>E. lata</i>	33
	<i>F. mediterranea</i>	26
	<i>D. seriata</i>	18
	<i>P. aleophilum</i>	5
	<i>P. viticola</i>	3
Sectorial necrosis of hard consistence	<i>E. lata</i>	67
	<i>D. seriata</i>	33
	<i>P. chlamydospora</i>	17
	<i>P. viticola</i>	1
	<i>Phomopsis sp.</i>	1
	<i>F. mediterranea</i>	1
Brown stripe	<i>D. seriata</i>	32
	<i>E. lata</i>	11
	<i>P. chlamydospora</i>	5
Punctuations in the healthy wood	<i>P. chlamydospora</i>	53
	<i>D. seriata</i>	20
	<i>E. lata</i>	7
Sectorial brown fan-shaped necrosis	<i>P. chlamydospora</i>	67
	<i>E. lata</i>	33
Brown to grey-brown necrotic area and brown-orange punctuations	<i>D. seriata</i>	82
	<i>P. chlamydospora</i>	6
	<i>S. hirsutum</i>	6

decay of most of the vinestocks, which made it difficult to find healthy wood at the border of the necroses.

Conclusion

The microbiological isolations in the wood confirmed the visual observations of the foliar symptoms, namely the presence of *D. seriata* responsible for BDA symptoms, and *P. chlamydospora*, one of the precursors of esca. In that, the isolations made revealed the same fungi as in the other french vine-growing areas, except the quite absence of *P. aleophilum*, which is the case for the Champagne too, and no detection of *N. parvum*. The presence of *E. lata* in the vinestocks of one of the investigated plot, is linked to the observation of foliar symptoms of this disease. The high isolation frequency of *D. seriata* as quite unique fungus in one to six-year-old diseased wood in sectorial grey-brown necrotic areas or brown-orange punctuations, may be related to the severe damages observed on the vegetation.

It is interesting to note that these vineyards never treated with sodium arsenite show now an upsurge of the disease, especially BDA, like other numerous vineyards in Al-

sace, in contradiction with the situation in close Germany, where BDA is not reported yet, but in agreement with the general situation in France.

Now, the aim of further investigations should be to know what, if cultural practices, variety sensitivity or climatic conditions, or a combination of it, are the reasons for such differences.

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