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Observation on black dead arm in French vineyards

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Summary. Black dead arm was first identified in French vineyards in 1999. It produces symptoms in the vine wood, causing decline and eventually death. Previously, this disease was confused with esca because of the similarity of the foliar symptoms. Two forms of black dead arm, a mild and a severe form, were observed in French vineyards on Cabernet Sauvignon and Sauvignon. Isolations from 23 vines collected from four vineyards located in three viticultural districts of Bordeaux showed that the main fungi associated with the brown streaks were *Botryosphaeria obtusa*, *B. dothidea* and *Trichoderma* spp. Pathogenicity tests showed that *B. obtusa* and *B. dothidea* caused dark lesions on 1-year-old canes. Therefore, these two fungi merit further study to determine their role in causing black dead arm.

Key words: grapevine, *Botryosphaeria obtusa*, *Botryosphaeria dothidea*.

Introduction

Black dead arm is a cryptogamic disease that was described for the first time in 1974 from Hungarian vineyards (Lehoczky, 1974), and later from Italy in the provinces of Isernia (Cristinzio, 1978) and Reggio-Emilia (Rovesti and Montermini, 1987). The disease produces symptoms in the wood of the plant, causing decline and eventually death.

The term black dead arm was introduced by Lehoczky (1974) to distinguish the disease he observed from the dead arm disease caused by *Phomopsis viticola* Sacc. The cause was attributed to *Botryosphaeria stevensii* Shoem. (anamorph *Diplodia mutila* Fr. apud Mont.) in Hungarian vineyards (Lehoczky, 1974), and to *Botryosphaeria obtusa* (Schwein.) Shoem. [anamorph *Sphaeropsis malo-*

rum (Berk.) Berk.] in Italian vineyards (Cristinzio, 1978; Rovesti and Montermini, 1987).

Recently, this disease was identified for the first time in France, in a Bordeaux vineyard (Larignon *et al.*, 2000). In the past, the disease was confused with esca because of the similarity of the foliar symptoms. Annual losses in Bordeaux vineyards from black dead arm are estimated to be from 4 to 20%.

In the Bordeaux area, affected vines over 8-years-old started to show symptoms by the end of May. Symptoms first appeared on the leaves at the base of the shoots and then spread to the other leaves, continuing to appear throughout the growing season.

Two forms of the disease were observed: a severe form and a mild form, leading in each case to premature leaf fall. The severe form (Fig. 1a) was characterized by dieback of one or more shoots, accompanied by leaf drop, shrivelling and drying of inflorescences or fruit clusters. Frequently, a few leaves remained clinging to the shoot tip (Fig. 1b).

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Sometimes, new growth occurred from axillary buds in the proximal portion of the shoot. In severe cases diseased shoots died entirely. The mild form was characterized by wine-red (red cultivars) or yellowish-orange (white cultivars) spots on the margins of the leaves or on the leaf blade, which coalesced to form large zones of deterioration between the veins and the margins of the leaf (Fig. 2 and 3). Inflorescences or fruit clusters may wither. Leaves that did not drop showed various patterns of necrosis which have been recently described by Larignon and Dubos (2001). In the wood, this disease was characterized by a brown streak 1–2 cm wide under the bark which began at the base of the affected shoot and extended upward to the graft union (Fig. 4). The brown streak is delimited by a yellowish-orange border a few millimeters in depth, in which the vessels are occluded.

This disease differed from esca in a number of characteristics. Thus, symptoms of black dead arm appeared earlier in the season (end of May) than those of esca (end of June). Leaves of vines with black dead arm never showed yellow spots. On red varieties, the red pigmentation was darker than that of esca. Brown streaking of the wood under the bark was observed only in black dead arm.

The purpose of this study was to identify the fungi associated with black dead arm in Bordeaux vineyards and to determine their pathogenicity.

Materials and methods

Plant material and fungal isolation

Twenty-three diseased grapevines of various cultivars were collected in 1998 and 2000 from four vineyards located in three viticultural districts of Bordeaux. Seven vines were collected at Medoc 1 from one plot each of Cabernet Sauvignon, Cabernet Franc and Merlot. At Medoc 2, seven vines were sampled from a plot of Cabernet Sauvignon and one of Sauvignon; at Entre-Deux-Mers, five vines from a plot of Cabernet Sauvignon, and at Sautes, four vines from a plot of Sauvignon. The vines ranged in age from 8–35 years and all had the foliar symptoms of black dead arm both (mild and severe form).

Isolations were made from brown streaks in the

wood of the trunks as described by Larignon and Dubos (1997). The fungi found were identified and the number of brown streaks infected with each fungus was recorded. Any *Botryosphaeria* species isolated were identified with reference to Shoemaker (1964), Lehoczky (1974), Phillips and Lucas (1997), and Punithalingam and Waller (1973).

Pathogenicity tests

Ten isolates of *Botryosphaeria obtusa* and three isolates of *Botryosphaeria dothidea* (Moug. : Fr) Ces. and De Not. were isolated from diseased vines collected in 1998 and 2000. They came from four of the vines previously mentioned (isolates marked with an asterisk in Table 1) and other vines from French regions (Table 1).

The ability of isolates of *B. obtusa* and *B. dothidea* to produce brown streaks was determined by inoculating them onto 1-year-old canes (10 cm long) of Cabernet Sauvignon in the laboratory. A hole 5 mm in diameter was drilled to the pith and filled with a 5-mm diameter plug cut from the growing edge of 2-day-old colonies on malt extract agar. Control canes were filled with uncolonized malt extract agar disks. Filled holes were sealed with Parafilm.

Twenty canes were inoculated with each isolate of *B. obtusa* and incubated for 21 days at 25°C in the dark. After this time, the bark was removed from the part of the cane around the hole and the length of any brown streaks was measured. Ten canes were inoculated with each isolate of *B. dothidea*, incubated for 7 days under the same conditions as described above, and examined for lesions as with *B. obtusa*. At the end of the experiment, pieces of tissue from symptomatic wood were transferred to plates of malt extract agar to test for *Botryosphaeria*. The experiment was repeated once.

Data were analyzed using the STATISTICA program (StatSoft, Tulsa, OK, USA) and the means of lesion lengths were compared using the Newman-Keuls test after analysis of variance (Snedecor and Cochran, 1957).

Results

Fungal isolation

The fungi most frequently isolated from the brown streaks were *Botryosphaeria obtusa*, *B.*



Fig. 1. Symptoms of the severe form of black dead arm on Cabernet Sauvignon. a) Dieback of shoots. b) After premature leaf fall, a few leaves remain clinging to the vine.

Table 1. Sources of *Botryosphaeria obtusa* and *Botryosphaeria dothidea* used for pathogenicity tests.

<i>Botryosphaeria</i> species	Isolate code ^a	Geographic origin	Source ^b	Cultivar	
<i>B. obtusa</i>	F98-1	Perpignan	SD (wood)	Syrah	
	F99-4*	Medoc	BDA (pycnidia)	Merlot	
	F99-7	Die	YGD (wood)	Clairette	
	F00-7	Champagne	BDA (streak)	Pinot Meunier	
	F00-14	Champagne	BDA (streak)	Pinot Meunier	
	PC R51 P178	Graves	BDA (pycnidia)	C. Sauvignon ^c	
	L VLC R8 P54*	Medoc	BDA (pycnidia)	C. Sauvignon	
	LL R12 P84*	Medoc	BDA (pycnidia)	C. Sauvignon	
	NJP R2 P75	Entre-2-Mers	BDA (pycnidia)	C. Sauvignon	
	NJP R5P5*	Entre-2-Mers	BDA (pycnidia)	C. Sauvignon	
	<i>B. dothidea</i>	Bd 1	Medoc	Berry	Merlot
		Bd 3	Champagne	BDA(streak)	Chardonnay
Bd 3-3		Medoc	BDA (wood)	C. Sauvignon	

^a Isolates are from the collection of Unité Mixte de la Recherche en Santé Végétale, INRA Bordeaux, France, except from (*) which were obtained from four vines examined during this study.

^b BDA: Black Dead Arm, SD: Syrah Decline, YGD: Young Grapevine Decline⁽¹⁾.

^c C. Sauvignon, Cabernet Sauvignon.

⁽¹⁾At the general Assembly of the 2nd ICGTD meeting held in Lisbon 2001 it was unanimously decided that the disease will henceforth be called Petri disease.



Fig. 2. Left and right, foliar symptoms of the mild form of black dead arm on Cabernet Sauvignon.



Fig. 3. Foliar symptoms of the mild form of black dead arm on Sauvignon.



Fig. 4. Symptom in the wood. Peeling off the bark showing a brown streak.

dothidea and *Trichoderma* sp. (Table 2) which were isolated from 69.6, 34.8 and 39.1% of all vines respectively. Others fungi, isolated at lower frequencies, were *Alternaria* sp., *Aspergillus* sp., *Botryosphaeria stevensii*, *Epicoccum* sp., *Papularia* sp., *Penicillium* sp., *Pestalotia* sp. and *Verticillium cephalosporium* (Table 2).

The frequency of each fungal species isolated varied according to site. At Entre-Deux-Mers, *B. obtusa* was the only species found and at Medoc 1 it was the dominant species, although *Trichoderma* and *Aspergillus* species were here also isolated. At Medoc 2 and Sauternes, both *B. obtusa* and *B. dothidea* occurred with species from several oth-

Table 2. Fungi associated with black dead arm symptoms in vines taken from four different vineyards in the Bordeaux region of France.

Fungal species	Vineyard ^a				
	Entre-Deux-Mers	Medoc 1	Medoc 2	Sauternes	Total
<i>Botryosphaeria obtusa</i>	5	7	2	2	16
<i>Botryosphaeria dothidea</i>	0	0	5	3	8
<i>Trichoderma</i> spp.	0	3	5	1	9
<i>Botryosphaeria stevensii</i>	0	0	1	0	1
<i>Aspergillus</i> spp.	0	2	1	0	3
<i>Penicillium</i> spp.	0	0	1	0	1
<i>Pestalotia</i> sp.	0	0	0	1	1
<i>Verticillium cephalosporium</i>	0	0	0	1	1
<i>Papularia</i> sp.	0	0	0	1	1
<i>Epicoccum</i> sp.	0	0	1	0	1
<i>Alternaria</i> sp.	0	0	0	1	1
Number of vines examined	5	7	7	4	23

^a The data are the number of plants with the brown streak from which the fungi were isolated.

Table 3. Pathogenicity of *Botryosphaeria obtusa* on 1-year-old canes of Cabernet Sauvignon.

Isolate code	Mean lesion length (mm) ^a	Virulence group
Control	0.63 a ^b ± 0.59	1
F99-7	1.6 a ± 1.82	1
NJP R2 P75	2.65 a ± 1.67	1
PC R51 P178	11.55 b ± 3.86	2
LL R12 P84	14.28 c ± 4.31	3
NJP R5P5	14.28 c ± 3.6	3
F99-4	14.3 c ± 4.23	3
LVLC R8 P54	16.2 cd ± 5.09	4
F00-14	16.85 d ± 5.4	4
F00-7	18.23 d ± 5.13	4
F98-1	18.38 d ± 4.16	4

^a Data are means of forty replicates per treatment ± SD.

^b Means in the same column followed by the same letter are not significantly different ($P=0.05$) according to the Newman-Keuls test.

er fungal genera, and here *B. dothidea* was dominant. *Trichoderma* species occurred only in the two Medoc vineyards.

Pathogenicity tests

Isolates of *B. obtusa* caused a dark streak in 1-year-old canes (Table 3) and were re-isolated from all lesions. There were significant differences in the mean lesion lengths caused by the various isolates. Four distinct virulence groups were distinguished. Isolates F98-1, F99-7, F00-14, LVLC R8P54 formed significantly longer lesions after 21 days than did the other isolates. Isolates F99-7 and NJP R2P75 caused the smallest necroses, which were similar in length to those of controls.

Isolates of *B. dothidea* caused dark streaks on 1-year-old canes after 7 days (Table 4) and were re-isolated from all lesions. There were no significant differences in the lengths of lesions produced by the three isolates of *B. dothidea*. No *Botry-*

osphaeria species were isolated from the controls.

A few differences were observed between the symptoms of black dead arm in the wood (natural infection) and the lesions on the canes caused by artificial inoculation with the two *Botryosphaeria* spp. Lesions on the canes were darker than the brown streaks observed in standing vines. Moreover, the yellowish-orange zones which delimited the brown streaks were not observed on the canes artificially infected with *Botryosphaeria*.

Discussion

The microbiological examination of the 23 diseased vines suggested that two fungi were mainly associated with black dead arm in the Bordeaux region, namely, *B. obtusa* and *B. dothidea*. Pathogenicity tests showed that *B. obtusa* produced dark lesions on one-year-old canes. These results are in agreement with those of Cristinzio (1978), and Rovesti and Montermini (1987), who considered *B. obtusa* a pathogen of grapevines in Italy. However, various authors consider this fungus a saprophyte (Larignon and Dubos, 1997) or secondary pathogen (Phillips, 1998). Larignon and Dubos (1997) frequently found it in association with *Eutypa lata* and *Fomitiporia punctata* where it was considered a secondary fungus in the process leading to the wood degradation characteristic of esca (Larignon, 1991). Phillips (1998) showed that it was weakly pathogenic on grapevine. Its frequent association with *Phomopsis viticola* and *B. dothidea* suggested that it may be a secondary pathogen. These differences could be due to differences in virulence among pathogen strains since in our pathogenicity

tests, two of the isolates were not pathogenic.

Concerning *B. dothidea*, our study showed that this fungus may also be involved in black dead arm. These results corroborate those of Phillips (1998) who showed that *B. dothidea* caused dark lesions on 1-year-old vine canes. He also reported it as a pathogen of grapevine in Portugal where it was associated with excoriosis and dieback of grapevines. Paradelo Filho *et al.* (1995) also reported it as causing a trunk canker of grapevines in Brazil. Furthermore, it is well documented as a pathogen of other lignicolous plants such as thornless blackberry (Maas, 1984), pistachio (Michailides, 1991), almond (English *et al.*, 1975) and peach (Weaver, 1974).

Of the remaining fungi isolated from the streaks, only *B. stevensii* has been reported to be pathogenic on grapevine, causing black dead arm of grapevines in Hungary (Lehoczky, 1974). The other fungi are most likely saprophytic on the tissues killed by the parasites. *Trichoderma* spp., often isolated from brown streaks, are known to be antagonistic to many fungi (Dubos, 1987).

The data presented in this paper show that two fungi are associated with black dead arm in the Bordeaux area. However, the symptoms of black dead arm on the wood were not reproduced exactly when these fungi were inoculated on the cut canes. This is probably due to the fact that the tests were not done on standing vines. Inoculations into standing vines and reproduction of foliar symptoms would be necessary to determine the involvement of these fungi in the disease.

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Table 4. Pathogenicity of *Botryosphaeria dothidea* on 1-year-old canes of Cabernet Sauvignon.

Isolate code	Mean lesion length (mm) ^a	Virulence group
Control	0.8a ^b ± 0.25	1
Bd 1	53.7b ± 15.23	2
Bd 3	44 b ± 12.23	2
Bd 3-3	42.4b ± 12.63	2

^a Data are means of twenty replicates per treatment ± SD.

^b Means in the same column followed by the same letter are not significantly different ($P=0.05$) according to the Newman-Keuls test.

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