FUNGAL COMMUNITY ASSOCIATED WITH GRAPEVINE WOOD LESIONS IN LEBANON

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Abstract

Aims: To detect and identify the cultivable micro-organisms putatively associated with esca disease in representative Lebanese vineyards.

Methods and results: Two field surveys were conducted in Lebanon in 2005 and 2007 to study the fungal community associated with grapevine wood lesions. A total of 68 vines showing typical esca symptoms were randomly sampled in 17 vineyards and cross sections were obtained of cordons and trunks. The shape and type of inner necrosis and discoloration were examined and isolations were made from the symptomatic wood. Isolation results showed that inner necrosis and isolated fungi were similar to those previously found elsewhere, namely in Central Europe or Mediterranean countries. Additionally, three methods for numerical evaluation of micro-organisms found were compared.

Conclusion: Most fungal pathogens generally associated with grapevine trunk diseases were detected, of which the basidiomycete *Fomitiporia mediterranea* and species of the ascomycete family *Botryosphaeriaceae* were the most frequently encountered. Additionally, a large diversity of other wood colonizing micro-organisms was detected. The putative role of some of the obtained micro-organisms in the process of wood degradation related to esca disease is discussed.

Significance and impact of the study: This isolation study is presently the most completed that was carried out with grapevine wood samples collected in Lebanon. Besides, it is the first to provide isolation results based on a classification of inner necrosis in five categories and to compare three criteria for numerical evaluation. This study also tends to further highlight that *Botryosphaeriaceae* species are common wood inhabiting fungi that should be associated with esca.

Key words: *Botryosphaeria* dieback, eutypa dieback, esca, trunk diseases, *Vitis vinifera*

Résumé

Objectifs : Isoler et caractériser la microflore cultivable dans des ceps symptomatiques d'esca provenant de vignobles représentatifs du Liban.

Méthodes et résultats : Deux enquêtes ont été réalisées au Liban en 2005 et 2007 pour étudier la communauté fongique associée aux lésions du bois de vigne. Un total de 68 ceps montrant des symptômes typiques d'esca ont été choisis au hasard dans 17 vignobles. Des sections transversales de bois ont été prélevées à partir des troncs ou des bras pour effectuer des isolements selon une procédure classique. Le type de nécrose interne, sa forme et la couleur des lésions ont été notées. Les résultats ont montré que l'aspect des nécroses internes ainsi que les champignons isolés du Liban étaient similaires à ceux précédemment observés ailleurs, notamment en Europe centrale ou sur le pourtour méditerranéen.

Conclusion : La plupart des champignons pathogènes généralement associés aux maladies du bois de la vigne ont été détectés dans cette étude. Cependant le basidiomycete *Fomitiporia mediterranea* et des espèces de la famille *Botryosphaeriaceae* ont été les plus fréquemment rencontrés. Au-delà des parasites les plus connus, figurait également une large diversité de micro-organismes colonisant le bois. Le rôle de certains micro-organismes dans le processus général de dégradation du bois de vigne associé à l'esca est discuté.

Signification et impact de l'étude : Cette caractérisation de la microflore associée à l'esca dans des vignobles libanais est la plus complète existant à ce jour. Cette étude est aussi la première à présenter des résultats d'isolements à partir de nécroses classées en au moins trois grandes catégories : nécroses sectorielles, centrales et mixtes (et non deux usuellement). Cette étude tend aussi à démontrer une fois encore que les champignons du genre *Botryosphaeria* devraient être considérés comme des pathogènes de l'esca.

Mots clés: dépérissements à *Botryosphaeria*, eutypiose, esca, maladies du bois de la vigne, *Vitis vinifera*

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INTRODUCTION

Since the end of the 20th century, the economical impact of trunk diseases has become a limiting factor for grapevine production in many countries around the world (Armengol *et al.*, 2001). In adult vineyards, eutypa dieback, esca and *Botryosphaeria* dieback are considered to be the most destructive (Dubos, 2002; Mugnai *et al.*, 1999; Úrbez-Torres, 2011). These diseases are generally characterized by the development of typical inner necrosis into the grapevine wood and are assigned to infection by pathogenic fungi that deteriorate the perennial and vascular system.

In Lebanon, with an area of cultivation of about 10600 ha, grapevine is one of the most important fruit crops, ranking third after stone and pome fruits, and is distributed in four districts: Bekaa valley (70 %), North Lebanon (14 %), Mount Lebanon (11 %) and South Lebanon (5 %) (Anonymous, 2010). The grape industry has markedly grown in the last 10 years. The table grape cultivars Beitamouni, Tfaifihi and Biadi are the most important native table grape varieties currently grown in Lebanon, however, new vineyards planted almost exclusively with imported international wine grape varieties (Cabernet-Sauvignon, Merlot, Syrah, Cinsault, Sémillon, Ugni blanc and Chardonnay) have been largely established mainly in the Bekaa valley. Up to the beginning of the 21st century, there was little information about the incidence of grapevine trunk diseases in Lebanon. But in recent years, the incidence of esca and grapevine decline has increased, resulting in reduced vigour and productivity and finally the death of branches or whole vines. A preliminary survey done in the West Bekaa valley in 2004 on 11 vineyards of local table or wine grapes (Choueiri *et al.*, 2006) reported foliar symptoms of esca, eutypa dieback and black dead arm according to the description made by Larignon *et al.* (2001). However, it was later demonstrated that foliar symptoms attributed to black dead arm are in fact esca symptoms in their initial stage of development (Lecomte *et al.*, 2012). In our survey, esca was the most common disease encountered, whereas eutypa dieback was rare.

The expression of esca symptoms on the leaves is commonly associated with the development of different fungal pathogens in grapevine wood (Arnaud and Arnaud, 1931; Chiarappa, 1959; Dubos, 2002; Mugnai *et al.*, 1999). Cross sections of the trunk of diseased vines generally reveal a variety of lesions and/or decay types (Larignon and Dubos, 1997; Larignon, 2004; Maher *et al.*, 2012). The wood of older vines usually shows a white to yellow soft rot, from which basidiomycetes like *Fomitiporia* spp. (in Europe usually *F. mediterranea*; Fischer, 2002) may be isolated (Fischer and Kassemeyer, 2003). The wood lesions continue to extend as the vines age and dead tissue increases in volume and can cause, in severe cases, a sudden wilting of the canopy known as apoplexy (Mugnai *et al.*, 1999).

In the last two decades, the number of studies that have characterized grapevine wood-inhabiting fungi has increased. In the Mediterranean basin, previous studies had shown that vines affected with esca generally show the presence of the same range of phytopathogenic fungi, namely the mitosporic fungi *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* as well as the most frequent basidiomycete *F. mediterranea* (Armengol *et al.*, 2001; Aroca *et al.*, 2006; Larignon and Dubos, 1997; Luque *et al.*, 2009; Mugnai *et al.*, 1996; Péros *et al.*, 2008; Rumbos and Rumbou, 2001). Most of these studies pointed out the presence of many other fungi, of which *Botryosphaeriaceae* fungi are often reported. In the previous report done by Choueiri *et al.* (2006), the only identified fungi found to be associated with esca and other declines in Lebanon were *Fomitiporia* sp., *Pa. chlamydospora* and *Diplodia seriata* (“*Botryosphaeria obtusa*”).

The aim of the current study was to provide a more complete analysis of the grapevine wood microflora associated with trunk diseases in Lebanon. For this purpose, we report results of isolations from necrotic wood of 68 table or wine grapevines mostly collected in the Bekaa valley and showing external esca symptoms. The study included two surveys that started in September 2005 and September 2007, respectively. Usually, different criteria are used in the literature to express the frequency of isolation of cultivable micro-organisms in the wood; therefore, we compare three different criteria for numerical evaluation of micro-organisms.

MATERIALS AND METHODS

1. Field surveys

The study was based on two separate surveys. In September 2005 (Survey 1), 11 vineyards in the West and Central Bekaa valley were visited and 39 symptomatic adult vines (7-14 years old) showing typical esca foliar symptoms were randomly collected (Table 1). The cultivars ‘Cinsault’ (23 vines), ‘Syrah’ (6 vines) and ‘Ugni blanc’ (5 vines) were the most encountered. The vines were stored at 5°C, and then one to three wood disks transverse sections were collected from each vine, from the trunk or from the

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cordon. The obtained wood disks of approximately 4 cm in thickness were photographed and sent to INRA, Bordeaux, France for laboratory isolation. In September 2007 (Survey 2), 29 adult vines were collected in 6 vineyards (6-13 years old) located in West Bekaa and North Lebanon (Table 1). The cultivars Cabernet-Sauvignon (15 vines) and Sauvignon blanc (7 vines) were the most encountered. During this second survey, wood samples were mostly collected from the cordons. Again, samples were stored at 5°C, cut, photographed and sent for microbial analysis to INRA, Bordeaux, France.

The wood symptoms were categorized according to their shape or discoloration as described by Larignon (2004) and Liminana *et al.* (2009). Shapes were punctuations, necrotic lines, central necrosis, sector shaped necrosis and mixed (central and sectorial) necrosis. In addition, five categories of discolorations were used to characterize each lesion: white decay (amadou), white decay together with brown necrotic wood, brown necrotic wood, black spots (=punctuations) and black necrotic lines along a wood ring. Some examples of inner necrosis observed in cross sections are shown in Figure 1. The category was determined on the basis of the dominant discoloration; however, other types of wood symptoms could be present.

2. Isolation from grapevine wood tissues

Laboratory isolations were performed as described by Larignon and Dubos (1997). Depending on the diversity of the lesions observed, one to four samples (2 x 1 cm) per wood disc (1 disc/vine) were removed with a pruning shear. In Survey 1, wood tissues were mostly collected at the edge of each necrosis, whereas in Survey 2 they were mainly collected from the necrotic tissues. This resulted in a total number of 97 wood samples for Survey 1 and 52 wood samples for Survey 2. Twenty wood chips of about 10 mm^3 were cut from each sample, representing a total of 1,940 (97 x 20) and 1,040 (52 x 20) wood chips for Survey 1 and 2, respectively. Chips were disinfected by immersion in active chlorine (3 % Na_2ClO_3) for 30 s and plated on malt extract agar (MEA). Petri dishes were incubated up to one month and were regularly checked for the development of micro-organisms. Identification of the developing fungal colonies was at the species or at least genus level. Besides, the presence of yeasts or bacteria, although not identified, was noted.

In a first round, identification of the fungi was based on cultural and morphological characters. Those generally considered as trunk disease pathogens were compared with the following local or international reference isolates: *Fomitiporia* sp. (F85-1), *Pa.*

Table 1. Origin of the vines collected in Lebanese vineyards in the summers of 2005 and 2007

Year	Vineyard location		Age	Cultivar (number of vines examined)
	Region	Site		
2005	West Bekaa	Aana	7	Cinsault (4)
"	"	Ammiq	14	Cinsault (3)
"	"	"	12	Tfaifihi (1)
"	"	Mansoura	13	Cabernet-Sauvignon (2)
"	"	Khirbet Qanafar	14	Chardonnay (1)
"	"	"	10	Merlot (1)
"	"	Kefraya	10	Cinsault (7)
"	"	"	9	Syrah (6)
"	"	Mansoura	11	Cinsault (1)
"	Central Bekaa	Taanayel	10	Cinsault (8)
"	"	"	11	Ugni blanc (5)
2007	West Bekaa	Khirbet Qanafar	10	Sémillon (1)
"	"	"	6	Sémillon (1)
"	"	"	8	Sémillon (1)
"	"	Mansoura	13	Sauvignon blanc (7)
"	"	"	13	Cabernet-Sauvignon (15)
			6	Cabernet-Sauvignon (2)
"	North Lebanon	Kfifan	7	Syrah (1)
			8	Grenache (1)

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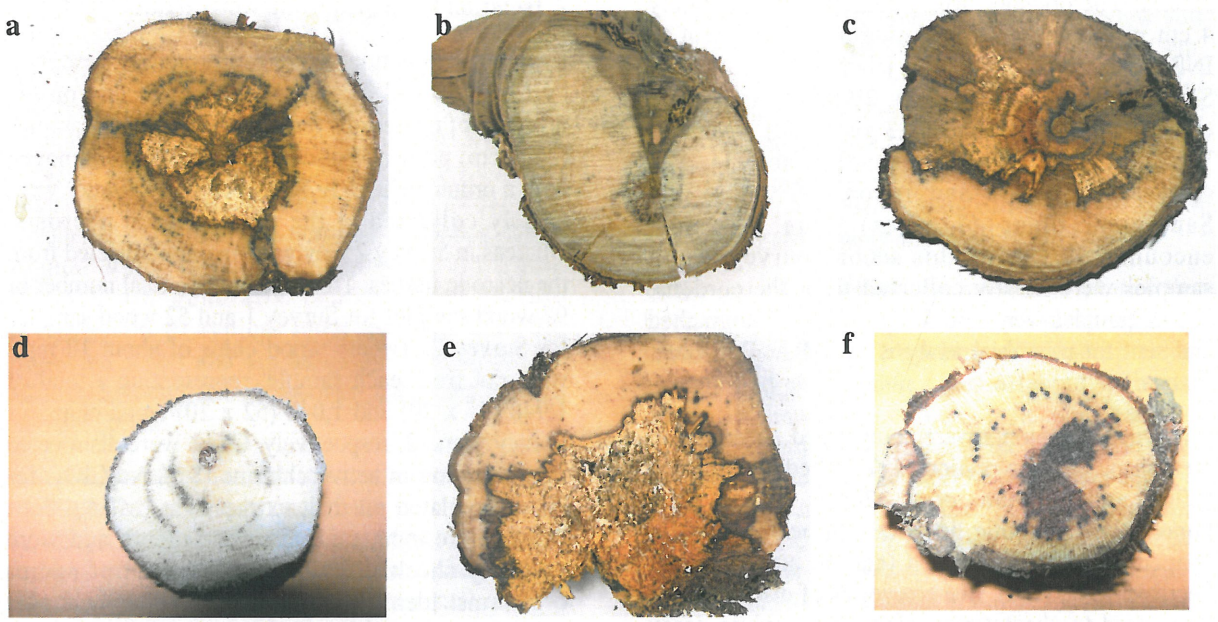

Figure 1. Examples of inner necrosis from trunk sections associated with trunk diseases and characterized according to their shape or discoloration: a: central lesion with "amadou" (white rot), surrounded by brown lesions and punctuations; b: wedged lesion and some punctuations; c: mixed lesions with "amadou" and brown sectors; d: necrotic lines; e: mixed necrosis with white rot, brown lesion and black necrotic lines; f: black spots or punctuations and dark or brown lesions.

chlamydospora (AQ04) (Comont *et al.*, 2010), *Pm. aleophilum* (CO-47), *Eutypa lata* (BX1-10) (Lecomte *et al.*, 2000), *D. seriata* (Ds10) (Laveau *et al.*, 2009), *Neofusicoccum parvum* (Np8) (Laveau *et al.*, 2009), *D. mutila* (CBS 112.553), and *B. dothidea* (CBS 110.302). Reference isolates were either obtained from the Centraalbureau voor Schimmelcultures (the Netherlands) or from the collection of INRA Bordeaux laboratory in France. Among the isolates of *Fomitiporia* sp. found in this study, 11 were sent to Germany for identification at the species level by ITS sequencing (Fischer, 2002). Putative saprobes were identified only at the genus level according to Barnett and Hunter (1998).

Results of isolations are given as percentages of microbial recovery. Because several organisms may develop from the same infected wood chip (Larignon and Dubos, 1997), the total number of observations was higher than the total number of wood chips plated (total number of observations equal to total number of sterile wood chips plus the total number of wood chips yielding a given micro-organism). In accordance with the system introduced by Larignon and Dubos (1997), we used and compared the following three criteria: **A** = number of wood chips infected by a micro-organism compared with the total number of wood chips plated; **B** = number of wood chips that yielded a micro-organism compared with the total number of wood chips infected; **C** = number of wood chips that yielded a micro-organism compared with the total number of micro-organisms observed. For each survey, the correlation between the distributions of isolation frequencies provided by criteria A and C was analyzed by a Spearman correlation test (P=0.001). Data provided by the three criteria A, B and C for fungi considered as pathogens were compared by a Kruskal-Wallis test (P=0.05). Statistical analyses were performed using the software Statbox Pro 5 (Grimmersoft®). Moreover, the frequency of vines infected by fungi presently considered as trunk pathogens was also calculated.

RESULTS

1. Necrosis assessment

For both surveys, a similar pattern was observed about the distribution of the different shapes of inner necrosis recorded in the transverse wood sections. Mixed lesions (36 % and 29 % in Survey 1 and Survey 2, respectively) and sector-shaped lesions (32 % and 33 % in Survey 1 and Survey 2, respectively) were the ones most frequently found. As for the different discolorations observed in the wood sections, brown necrotic wood (50 %) and

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Table 2. Micro-organisms identified in Lebanese vineyards and relative percentages of isolation

	Survey 1 (2005-2006)			Survey 2 (2007-2008)		
	A[1]	B[2]	C[3]	A[1]	B[2]	C[3]
Fomitiporia mediterranea	**16,3**	**27,2**	**16**	**10,4**	**12,5**	**9,5**
Phaeomoniella chlamydospora	**11,2**	**18,6**	**10,9**	**8,7**	**10,5**	**7,9**
Phaeoacremonium aleophilum	**12,7**	**21,1**	**12,3**	3	3,6	2,7
Diplodia seriata	**4**	**6,7**	**3,9**	**5,9**	**7**	**5,3**
Neofusicoccum parvum	Not identified*			**6**	**7,1**	**5,4**
Botryosphaeria dothidea	Not identified*			1	1,2	0,9
Diplodia mutila	Not identified*			0,8	0,9	0,7
Other *Botryosphaeriaceae* fungi	**5,4**	**8,9**	**5,3**	**4,1**	**5,1**	3,9
***Eutypa* sp.**	0,9	1,5	0,9	1,9	2,3	1,7
***Phomopsis* spp.**	0,1	0,2	0,1	-	-	-
***Ilionectria* sp.**	0,3	0,5	0,3	-	-	-
Total pathogens	50,9	84,7	49,7	41,8	50,2	38
***Alternaria* sp.**	2	3,4	2	**10,7**	**12,8**	**9,7**
Aspergillus sp.	0,1	0,2	0,1	0,9	1,1	0,8
Aureobasidium sp.	0,2	0,3	0,1	0,4	0,5	0,3
Cephalosporium sp.	-	-	-	0,4	0,5	0,3
Chaetomium sp.	-	-	-	0,5	0,6	0,4
Cladosporium sp.	0,2	0,4	0,3	1,6	2	1,5
Epicoccum sp.	0,3	0,5	0,3	1,3	1,6	1,2
Fusarium sp.	-	-	-	1,1	1,3	1
***Gliocladium* sp.**	0,1	0,1	0,1	**6,6**	**8**	**6**
Nigrospora sp.	-	-	-	0,2	0,2	0,2
Paecylomyces sp.	-	-	-	0,1	0,1	0,1
***Penicillium* sp.**	0,8	1,3	0,7	3,7	**4,4**	3,3
Pestalotiopsis group	0,6	1	0,6	1,3	1,6	1,2
Trichoderma sp.	0,1	0,1	0,1	0,2	0,2	0,2
Yeasts or Bacteria	0,5	0,9	0,5	2,4	2,9	2,2
Spadicoides sp.	-	-	-	0,1	0,1	0,1
Stigmella sp.	-	-	-	0,5	0,6	0,4
***Ulocladium* sp.**	-	-	-	**4**	**4,9**	3,7
Other fungi, not identified	6,6	11	6,4	15,6	18,7	14,2
Total fungal saprobes	11	18,3		65,9	59	44,6
Yeasts or Bacteria	0,5	0,9	0,5	2,4	2,9	2,2
No micro-organism yielded	40		39,1	16,7		15,2
Total	102,4	103,9	100	110,1	112,1	100

in bold: fungal species that are presently considered either as grapevine trunk pathogens or fungal saprobes for which isolation percentages were higher than 4 %.

* These fungal species were supposed to be isolated in 2005 but were not identified and recorded precisely. They were included in the category "Other *Botryosphaeriaceae* fungi".

[1] Percentage of wood chips infected by a micro-organism out of the total number of wood chips plated. Based on 1,940 wood chips in Survey 1 and 1,040 wood chips in Survey 2. One wood chip may yield several micro-organisms.

[2] Percentage of wood chips infected by a micro-organism out of the total number of wood chips that yielded a micro-organism. Based on 1,164 wood chips in Survey 1 and 866 wood chips in Survey 2.

[3] Percentage of wood chips that yielded a micro-organism out of the total number of observations (total of micro-organisms observed plus sterile wood chips). Based on 1,986 observations in Survey 1 and 1,145 observations in Survey 2.

black spots (21 %) were most frequently encountered in Survey 2 (where samples had been taken mainly from the cordons), whereas white rot was more common in Survey 1 (25 %) (where samples were mainly collected from the trunk) compared to Survey 2 (6 %). The occurrence of white rot associated with brown lesions was identical (19 %) in both surveys.

2. Isolation

In each survey, a variety of micro-organisms, pathogens or saprobes was often co-isolated from affected wood tissue. In Survey 1, of the 97 wood samples analyzed, only two did not yield any fungal pathogen and 15 yielded only one single pathogenic taxon. In Survey 2, of the 52 wood samples examined, 15 did not yield any fungal pathogen and one yielded only one single saprophytic species. In Survey 1, the 1,940 wood chips that were plated on MEA produced 1,986 microbial colonies. A total of 776 wood chips (40 % out of 1,940 plated chips) did not yield any fungal micro-organism and a total of 1,164 wood chips were infected, resulting in the development of 1,210 colonies. In Survey 2, the 1,040 wood chips produced 1,145 microbial colonies. In total, 174 chips (16.7 % out of 1,040 plated chips) did not yield any microbial organism while 866 were found infected and produced 971 colonies. In both surveys, the presence of yeasts or bacteria, although not identified, was noted.

As shown in Table 2, the main pathogenic wood fungi isolated from the wood chips were *F. mediterranea* (selected isolates of *Fomitiporia* sp. were all identified as *F. mediterranea* by ITS sequencing), *Pm. aleophilum*, *Pa. chlamydospora*, species of *Botryosphaeriaceae*, and *Phomopsis* spp. Many other fungi considered as fungal saprobes were also identified, of which the most frequent were *Alternaria* sp., *Gliocladium* sp., and *Penicillium* sp.

When comparing the distribution of isolation percentages in each survey, no significant difference was observed between the ranking provided by the three criteria (P=0.609 in Survey 1 and P=0.741 in Survey 2). Criteria A and C provided similar percentages in both surveys. The correlation between the percentages provided by criteria A and C was highly significant (P=0.000) in both surveys. These percentages showed that *F. mediterranea* was the most frequently detected fungal species, followed by *Botryosphaeriaceae* spp., *Pa. chlamydospora*, and *Pm. aleophilum*. *Eutypa* sp. and *Phomopsis* spp. were much less encountered. The distributions of the percentages of vines from which a grapevine pathogen was isolated also provided similar results in both surveys (Figure 2). The proportion of wood chips yielding a micro-organism compared to the total number of wood chips infected (Criterion B) allowed another comparison between surveys. In Survey 1, the percentage of wood chips infected by a fungal pathogen was much higher (84.7 %) than in Survey 2 (50.2 %). On the contrary, the percentage of

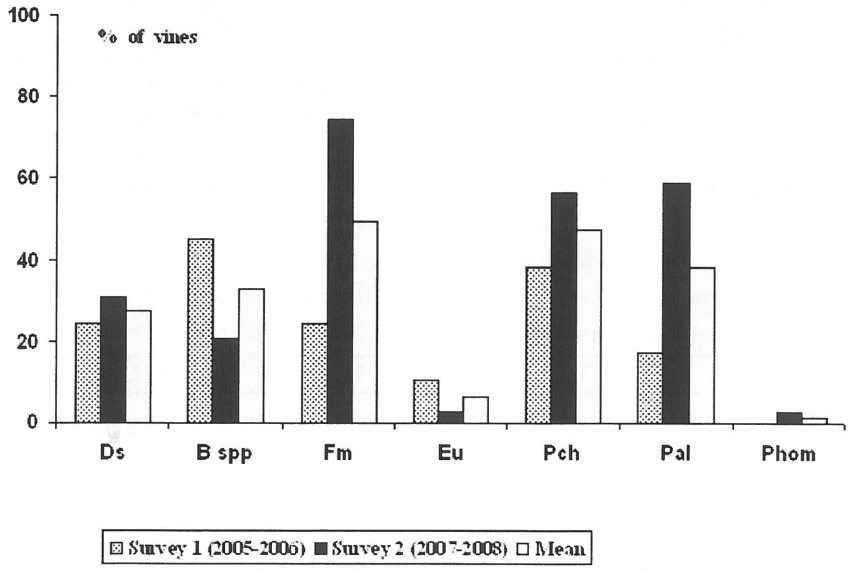

Figure 2. Percentage of vines from which a grapevine fungal trunk pathogen was isolated in Survey 1 (39 vines) and 2 (29 vines).

Ds: *Diplodia seriata*, B spp: other *Botryosphaeriaceae* species, Fm: *Fomitiporia mediterranea*, Eu: *Eutypa* sp., Pch: *Phaeomoniella chlamydospora*, Pal: *Phaeoacremonium aleophilum*, Phom: *Phomopsis* spp.

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Table 3. Percentage (criterion C) of wood chips infected by a fungal pathogen with regard to the shape of inner necrosis

Year of sampling	Shape of necrosis	Number of sample	Number of infected wood chips	Saprobes*	Fungal pathogens associated with grapevine wood necrosis						
					Fm	Bot	Eu	Pch	Pal	Phom	Total %
2005	Central	31	362	25,1	**29,8**	7,2	4,7	8,8	**24,3**	0	74,9
Survey 1	Sector-shaped	15	235	25,1	**24,3**	**43,4**	0	3,8	3,4	0	74,9
	Mixed	35	400	11,7	**35**	10,5	0	**15,5**	**26,8**	0,5	88,3
	Black spot	10	137	13,9	0,7	2,9	0	**78,8**	3,6	0	86,1
	Necrotic line**	6	76	17,1	**14,5**	10,5	0	7,9	**50**	0	82,9
2007	Central	7	114	47,4	**14,9**	11,4	0	**17,5**	8,8	0	52,6
Survey 2	Sector-shaped	17	367	70	0	**23,4**	5,2	0,8	0,5	0	30
	Mixed	15	291	27,1	**30,6**	**29,6**	0,3	5,8	6,5	0	72,9
	Black spot	11	165	70,9	1,2	0	0	**27,9**	0	0	29,1
	Necrotic line***	2	34	85,3	0	0	0	**14,7**	0	0	14,7

Fm: *Fomitiporia mediterranea*, Bot: *Botryosphaeriaceae* species, Eu: *Eutypa* sp., Pch: *Phaeomoniella chlamydospora*, Pal: *Phaeoacremonium aleophilum*, Phom: *Phomopsis* spp
In bold: percentages close to or higher than 15.
* Including saprophytic fungi, yeasts and bacteria; ** as shown in Figure 1e; *** as shown in Figure 1d.

saprobes was much lower in Survey 1 (18.3 %) than in Survey 2 (59 %). Isolation frequency of *Pm. aleophilum* was much higher in Survey 1 than in Survey 2.

3. Internal lesions and fungi associated with grapevine trunk diseases

Criterion C was used to compare in each survey the distribution of six wood inhabiting fungal pathogens with regard to the shape of inner necrosis (Table 3). *F. mediterranea* was mostly isolated from central and mixed necrosis in both surveys. Fungi of the *Botryosphaeriaceae* were frequently found in sector-shaped and mixed lesions. In both surveys a high percentage of recovery of *Pa. chlamydospora* was obtained from wood chips taken from black spot lesions, though it was also recovered from either mixed or central lesions, depending on the survey. *Pm. aleophilum* was recovered from necrotic lines and mixed or central lesions. *Eutypa* sp. was mostly detected from V-shaped and central lesions, depending on the survey, and *Phomopsis* spp. were only detected from mixed lesions in Survey 1.

As for the relationship between fungal pathogens and discoloration (Figure 3), *F. mediterranea* was isolated from all types of lesions in Survey 1 but mainly from white rot and/or brown lesion in Survey 2. Fungi of the *Botryosphaeriaceae*, including *D. seriata*, were frequently detected from brown lesions. In contrast to Survey 2, *Pa. chlamydospora* and *Pm. aleophilum* were recovered from all lesion types in Survey 1. *Pa. chlamydospora* was mainly recovered from necrotic lines or black spots. In general, *Eutypa* sp. and *Phomopsis* spp. were rarely isolated and were never found in necrotic lines, black spots or white rot. *Ilionectria* sp. was isolated in Survey 1 only and mostly from black spots.

DISCUSSION

The current study mainly describes the fungal community within grapevines affected by esca in Lebanese vine growing regions. Collections were made in 2005 and 2007 after the expression of typical esca leaf symptoms (Lecomte *et al.*, 2012). Isolations were performed in wood lesions classified into at least three main categories as described by Larignon (2004) or Maher *et al.* (2012). To our knowledge, this is the first study using this classification method before microbial examination. The importance of grapevine in the Lebanese economy and the presence of trunk decline diseases in this country (Choueiri *et al.*, 2006) have stimulated this survey. It also allowed us to observe the development of foliar esca symptoms (data not shown) during the summer of 2006 and to further demonstrate that leaf symptoms previously attributed to black dead arm (according to the description made by Larignon *et al.*, 2001) in Lebanon (Choueiri *et al.*, 2006) had been actually confused with those of esca (Lecomte *et al.*, 2012).

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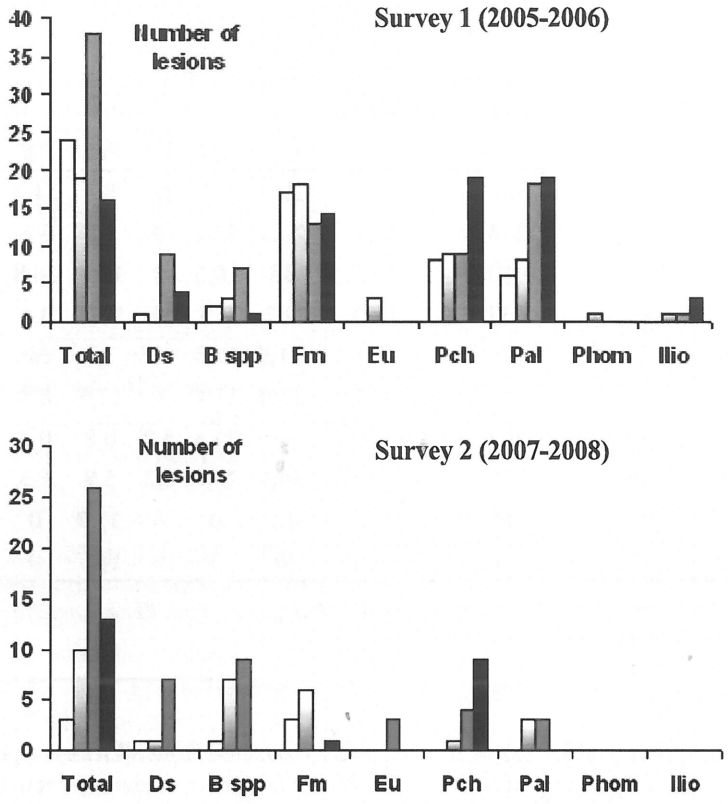

Figure 3. Number of lesions distributed according to their discoloration and from which a fungal trunk pathogen was isolated.

Ds: *Diplodia seriata*, B spp: other *Botryosphaeriaceae* species, Fm: *Fomitiporia mediterranea*, Eu: *Eutypa* sp., Pch: *Phaeomoniella chlamydospora*, Pal: *Phaeoacremonium aleophilum*, Phom: *Phomopsis* spp., Ilio: *Ilionectria* sp. White bars: white rot; white and grey bars: white rot and brown lesion; grey bars: brown lesion; black bars: necrotic lines and black spots.

In general, fungal identification made in France and Germany for both surveys yielded all the fungi already referred to in the literature as either causal micro-organisms of trunk diseases or at least often associated with grapevine wood (Larignon and Dubos, 1997; Péros *et al.*, 1999; Péros *et al.*, 2008). Although the percentage of wood chips that did not yield micro-organisms was different in the surveys, the diversity of the associated mycota was similar, with ascomycetes being most prominent. Several pathogens or saprobes were often co-isolated from the same wood sample, indicating that many of them can co-inhabit grapevine wood as suggested by Péros *et al.* (2008).

Three different approaches to calculate the frequencies of isolation were used in this study. Frequencies obtained by Criteria A and C were very similar, most likely because the total number of colonies observed was not much greater than the total number of wood chips. In such a situation, we recommend the use of only one of the above criteria. Criterion B was used to assess the relative proportion of each micro-organism among those detected, independently from the number of sterile wood chips. This allowed for a rapid comparison between surveys, especially in identifying the most frequent colonizing species. Differences in the percentages of healthy wood fragments, saprobes or pathogens between surveys may be explained by the origin of the samples. In Survey 1 the samples were taken mainly from the trunks and isolations were made from the border of lesions, while in Survey 2 samples were mainly taken from the cordons and isolations were made close to the margin but essentially within the lesions.

The three pathogenic species generally associated with esca in Europe (*F. mediterranea*, *Pa. chlamydospora* and *Pm. aleophilum*) were consistently found in our study and this is in agreement with other studies (Larignon and Dubos, 1997; Mugnai *et al.*, 1996). Among the pathogens, *F. mediterranea* was most frequently isolated, mainly

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in white rot or brown necrosis, confirming that in the Mediterranean regions this fungus can be largely involved in the whole process of wood deterioration (Sparapano *et al.*, 2000). As already reported by Mugnai *et al.* (1999) and Pollastro *et al.* (2000), *Pa. chlamydospora* was mostly isolated from black spots. *Pm. aleophilum* was rarely found in black spots or sector-shaped necrosis, but was more present in necrotic lines at the border of necrosis. This observation is different from previous studies by Larignon and Dubos (1997) and Mugnai *et al.* (1999). *Pm. aleophilum* was more frequently encountered in Survey 1 than in Survey 2, which might be due to the different origins of the samples as mentioned above, or to the age of the inner necrosis, supposedly older in the trunks.

Eutypa sp. was rarely detected in the present study. This low occurrence confirms that this fungus is less abundant in dry regions as was also suggested in Spain (Luque *et al.*, 2009) or in South Africa (van Niekerk *et al.*, 2006). Moreover, this fungus was much less frequently isolated from the V-shaped lesions than *Botryosphaeriaceae* species. *D. seriata* and *N. parvum* were the most often isolated species in the current study. *Botryosphaeriaceae* fungi were also frequently found from mixed necrosis typical of esca (Larignon, 2004; Maher *et al.*, 2012). As recently mentioned by White *et al.* (2011), the role of these latter species - which were largely predominant in the wood of esca-affected vines and which were mainly considered as saprobe on grapevine in the past (Gubler *et al.*, 2005; Larignon and Dubos, 1997; Úrbez-Torres, 2011) - in the development of esca is still unclear and needs to be further studied. *Phomopsis* spp. and *Ilionectria* sp. associated with 'excoriose' (Phomopsis dieback) and black foot diseases, respectively, were rarely found in our survey.

The trunk disease fungi found in this study were similar to the preliminary results reported in Lebanon (Choueiri *et al.*, 2006) and with the results reported in Europe and in other Mediterranean countries (Armengol *et al.*, 2001; Aroca *et al.*, 2006; Larignon and Dubos, 1997; Luque *et al.*, 2009; Mugnai *et al.*, 1996; Péros *et al.*, 1999; Péros *et al.*, 2008; Rumbos and Rumbou, 2001) as well as in Africa (Berraf and Péros, 2005; White *et al.*, 2011). The current study sampled from a larger area including the main grapevine growing areas such as West Bekaa, Central Bekaa and North Lebanon and the isolations were more comprehensive than those conducted by Choueiri *et al.* (2006). New reports for fungal species on grapevines include *D. mutila*, *N. parvum*, *B. dothidea* as well as *Pm. aleophilum*. Many unidentified yeasts and bacteria were also isolated, and could also contribute to the whole process of wood degradation. As already reported in some of the recent literature related to grapevine trunk diseases (Bruez *et al.*, 2012; Hofstetter *et al.*, 2012), the current study also demonstrates that a very large diversity of colonizers may be involved in the whole process of wood deterioration. Therefore, further research has to be done to assess their exact contribution to the different grapevine decline diseases.

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