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Fungal pathogens associated with grapevine wood lesions in Algeria

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Abstract

A field survey was conducted in Algeria to assess the state of dieback in some vineyards and identification the fungal pathogens associated with necrotic wood lesions. Field surveys conducted during spring seasons in 2006 and 2012 revealed that an average disease incidience was 25 % , the indice of disease severity varied of 0.5 - 2%, based on herbaceous symptoms. Some sections of wood cultivar, collected from four localities vine showed many kinds of necrosis, central sectoral(brown colour), necrosis sectoral(grey colour) and central light brown. Wood samples were collected and used for isolation and then placed on potato-dextrose (PDA) medium. The results showed that the presence of wood fungi mostly affected the vine trunk were isolated and the fungi were identified morphologically as *Botryosphaeria* species and *Fomitiporia mediterranea*.

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Introduction

In Algeria, grapevine occupied an important place during the French colonization, through its high quality production; cultivation was concentrated in the northern regions of the country especially in the center and west or where the climatic conditions are very favorable for to the growth. In 2012 based on statistics from the Ministry of Agriculture, vine cultivation was around100.200 hectares for 334.021 tons of grape production. In recent years the production has decreased, the bio aggressors and the Grapevine fanleaf virus (GFLV) were accused for the loss, but the research work conducted between 2003-2006 by Ammad (2006) provided additional information on the state of dieback in vineyards studied. A new symptomatology of herbaceous part as yellowing symptoms, reddening of leaf necrosis leaves, shredded leaves, partial and total dieback of vines was observed. In past decade many studies have been conducted to identify the problem that threatens the sustainability of the vineyards. These studies were based on species identification, distribution and pathogenicity, the results of these studies showed the presence of many diseases. Among them, Esca, the most easily destructible part of the grapevine, leads to decline and loss of productivity of vines. In some areas of Mediterranean region more than 50% of the vineyards have disease incidence of 20 -30 % (Coretsi *et al*, 2000). The symptomatology of esca is considered to be caused by the basidiomycetes and genera of fungi that have been reportedto be associated with esca were Phaeomoniella, Fomitiporia, Phaeoacremonium, Stereum (Larignon and Dubos, 1997; Mugnai *et al*, 1999; Surico *et al*, 2006(Ammad *et al*., 2014).

Another disease affecting vines was *Botryosphaeria* canker, also known as Black Dead Arm, 'Bot' canker, Excoriose, Grapevine decline syndrome, *Diplodia* cane dieback and bunch rot (Savocchia *et al*, 2007 and Úrbez-Torres, 2011). First described in Hungary in 1974, *Botryosphaeria* canker has been reported in Australia, Italy, the United States, and Canada. The causal agent produces cankers on a wide range of woody plants. 'Bot' canker affects spurs, cordons, and trunks causing dieback of the grapevine (Rovesti *et al*, 1987).

A recent survey provided an evidence of that foliar symptom attributed to BDA in fact belonged to the esca syndrome (Lecomte *et al*, 2012). The Botryosphaeriaceous fungi can be localized in healthy tissue of all parts of plants and mostly cause disease after stress to trees or fruit after harvest and higher rainfall (Johnson *et al*, 1991, 1992); they develop into the wood and caused a decline and death in the end. Symptoms generally associated with *Botryosphaeria* species on grapevines include mortality, dieback, cankers and sector–shaped lesions inside the wood, bud mortality, reduced growth and in some cases bunch rot, while leaf spots and various other fruit, shoot and trunk diseases are common on other hosts also (Van Niekerk, 2004). Most studies have indicated that the vines affected by BDA generally showed the presence of phytopathogenic fungi, namely fungus *Botryosphaeria sp as Botyosphaeria viticola, B. Dothidae, B.corticola, Diplodia seriat*a and *Nesofusicoum parvum* of these species are often reported.

Cross and longitudinal sections through the canker area usually shows a variety of lesions and/or decay type (Larignon, 2004). In particular, the wood of older vines usually shows white to yellow soft rot, from which basidiomycetes like *Fomitiporia spp*. (in Europe usually *F. mediterranea*) was isolated (Fischer and Kassemeyer, 2003).

In this study an epidemiological survey was carried out to explore the disease that affect the vines in Algeria and the impact of the causative agents on the plant. Plants in 17 vineyards located in four regions knowed by these viticultural vocation : Medea (2 vineyards) Blida (8 vineyards) Tipaza (5 vineyard), Mostaganem (4 vineyards) were studied. The aim of this study was to i) further investigate the incidence of dieback in many vine localities, ii) observe the different types of inner necrosis into the grapevine trunks, iii) identify and characterize the causative

agents of grapevine dieback in Algerian vineyards from symptomatic vines.

Material and methods

The study was conducted during the period during the spring season in 2006 and 2012 in four vines areas: Tipaza Mostaganem, Blida and Medea, planted with different cultivars: Dattier de Beyrouth, Gros noir, Cabernet Sauvignon, Muscat, Cardinal, Carignan,Cinsault, Merlot and Syrah. These areas were situated in bio-climatic zone with an average annual precipitation of 600 mm.Vines were 5- 45 years old and were not treated with sodium arsenite or any other fungicide. All vines in each vineyards were inspected for dieback symptoms disease incidence and for disease severity. "According to Dubos (2002)" the random selection of the samples is the most objective way to select the stocks to watch and analyze. In each of the 17 vineyards studied, we randomly selected 500 vines avoiding border lines in 10 fragmented units where each unit is represented by 50 vines. Thus, the total of our observations concerned 8500 vines representing the four regions. Foliar symptoms generally consist of : small leaves compared to their normal state, distorted, sometimes chlorotic and necrotic,shoots have very short internodes, Wood samples were collected from branches of grape vine with dieback symptoms, including dead shoots, cankers, discolored tissues with many position or/ and several texture.We collected information that correspond with the states of decline, including the age of the vines, modes of conduct, phytosanitary treatments performed, in climatic conditions as shown Table 1.

Table 1. Characteristics of grapevines and grape regions surveyed in Algeria.

Locality	*Médéa*			*Blida*								*Tipaza*					*Mostaganem*	
site	Benchicao			Mouzaia								Hamr El Ain					ONCV	
Vine yards	1	2	1	2	3	4	5	6	7	8	1	2	3	4	5	1	2
Cultivar	Dattier de Beyrouth	Carignan	Cinsault	Dattier de Beyrouth	Cardinal	Carignan	Syrah	Cabernet sauvignon	Merlot	Cinsault	Dattier	Gros noir	Cinsault	Syrah	Cabernet sauvignon	Muscat	Muscat
Age (year)	26	45	11	10	12	12	05	10	09		40	10	12	11	11	6	30
Area (Ha)	12	06	10	6.5	05	05	04.5	04	03	06.5	05	04	03	04.5	03	10	11
Type of Pruning	Guyot simple	Guyot double	Guyot	Cordon double	Cordon double	Guyot	Guyot	Guyot	Guyot	Guyot	Cordon double	Cordon double	Guyot	Guyot	Guyot	Goblet	Goblet
Rootstock	41B	41B	41B	41B	41B	41B	SO4	SO4	41B	99R	41B	41B	SO4	99R	41B	41B	41B
Time of pruning	December																

ONC V National (Algerian) Office of Marketing of Wine Products

Disease assessment

The objective of our epidemiological survey was to focus on the detection of typical decline symptoms in each vineyard. The method used to assess the impact of decline disease on each vine consists of an evaluation of the sanitary status of each vine according to a gradual ranking scale using five classes as follows: no symptoms = class 1, symptoms on herbaceous organs (Leaf necrosis, stunting and / or foliar destruction)= class 2, partial dieback = class 3, total decline class 4 and dead vines = class 5. The incidence of decline (I) allows to assess an overall potential damage in each vineyard. This index is calculated using the following formula (Pollastro *et al*, 2007): $I (\%) = \Sigma\text{-}ni.i/N.v \times 100$, where ni is the number of vines receiving the note i (i = 1-4), N is the total number of vines affected by the decline disease in the vineyard surveyed and v is the highest value of the rating scale. The severity of the disease (G) allowed knowing the average value of the class of decline. This index was estimated by the following formula: $G = \Sigma (ni.i) / N$. All vines were also inspected in order, to observe the different development of inners necrosis. Infected samples with visible symptoms were transported to the laboratory for isolations.

Isolations from grapevine wood tissues

Ten wood chips were cut from each 10cep diseased grape vine of different cultivars had external disease symptoms on the foliage, obtained from viticultural regions visited during this survey, representing a total of 340 (10 ×10 ×17). Cross and longitudinal sections of the trunk of each vine were examined in order to

follow development of necrosis in the trunks, and the type of necrosis was recorder. Isolations were made from the different zones of necrosis tissue and from the margin of all necrosis categories at the frontier of necrotic tissues and apparently healthy, wood slices 0, 5 mm were surface disinfected in calcium hypochlorite (3 % NaOCl) for 4 min, rinsed with sterilized water and dried onto sterilized filter paper. Then, they were placed on potato-dextrose (PDA) plates and stored at 25°C. Observations of fungal development were recorded at weekly intervals, and the fungi isolated from wood were identified with the references. Results were given as a frequency of necrotic pieces infected with each microorganism for each zones of necrosis tissue.

Statistical analysis

Variance analyses and principal component analysis (PCA) associating data concerning regionsand disease incidence were performed using the Past software (version 6.0).

Results

Disease data and inner necrosis

The incidence of wood disease were evaluted on vines showing dieback symptoms varied depending upon region and cultivar. The average incidence of symptomatic plants at all vineyards prospected was 25.1%. The highest levels were in Medea, Blida and Mostaganem vineyards for which percentage of disease expression were respectively 18.1%, 24.4% and 25.5 % in 2006 and 33.4%, 32.9% and 33.0% in 2012 respectively. The results of our epidemiological investigations carried out in this study demonstrated the high incidence and severity of trunk diseases in Algerian vineyards. Wihle the incidence registered in Tipaza vineyards varied from 10.1 to 10.6 %. The severity of the dieback in vineyard obtained in all regions is ranged from 0.5 to 2%. 'Cabernet sauvignon', 'Sauvignon' and 'Syrah' appeared the most susceptible cultivars in this survey. Incidence of disease during the two seasons (2006 and 2012) revealed a moderate positive correlation (r = 0.70). The principal component analysis (PCA) was performed to visualize the position of the regions with respect to the two main axes. The first group (red) mainly takes in account the region of Blida. This group is positively correlated with disease incidence noted in 2012. The second group (green) is represented by regions Medea and Mostagnem and this group at the intersection of the two axes, is positively correlated with disease incidences for the two seasons. The third group (blue) included the region Tipaza in which no differences were observed according to (Figure 1). Similar results were noticed concerning the degree of severity of the disease in the four areas of study with a positive correlation as shown in (Figure 2). We also concluded that incidence and severity increased over time.

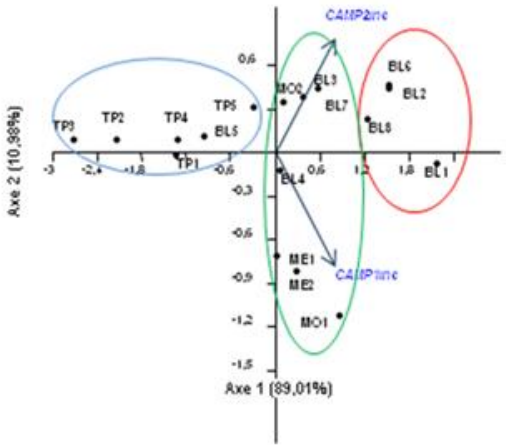

Fig. 1. Principal Component Analysis (PCA) of diseaese incidence.

CAMP1 INC: incidence enregistred in 2006, CAMP 2 INC: incidence enregistred in 2012.

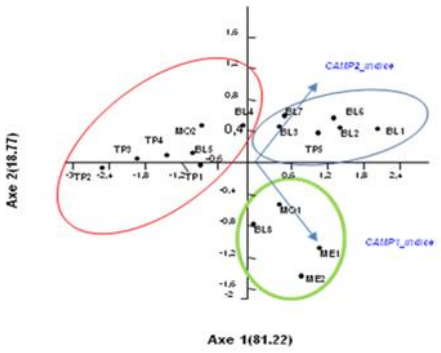

Fig. 2. Principal Component Analysis (PCA of disease severity.

CAMP1 indice in 2006, CAMP 2 indice in 2012.

MO1 : Mostaganem vineyard 1, **MO2** : Mostaganem vineyard 2(ONCV)

ME1 :Medea vinyards1, **ME2**: Medea vineyards2 , **BL1-BL2-BL3-BL4-BL5-BL6-BL7-BL8**: 8 vineyards in Blida, **TP1-TP2-TP3-TP4-TP5**: 5 vineyards in Tipasa

The analysis of the cross sections from trunks collected showed in (**Figure 3)** the presence of different forms of inner necrosis, that can be grouped into 4 main categories:(a) sectorial hard brown nécrosis,(b) light necrosis of soft consistency in sectorial position,(c) brown hard necrosis around light soft part and (d) complex necrosis (all form). In particular following necrosis was frequently encountered among the vines : the brown hard necrosis occurred in sectorial position with a clear and soft consistency necrosis in the center and inner necrosis appeared in all kinds of shape.

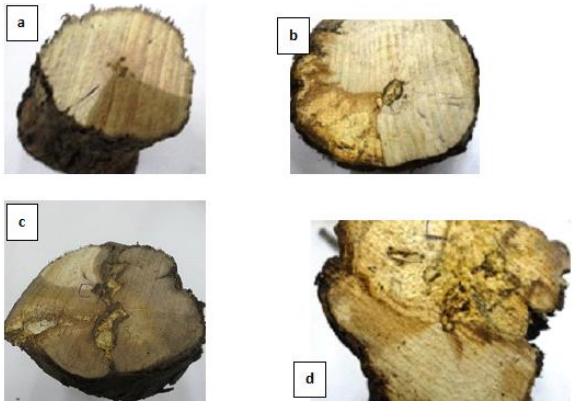

Fig. 3. Different form of necrosis: Cross sections (a,b,c) and longitudinal sections (d) of trunks associated with typical dieback symptoms on herbaceous parts: (a) sectorial hard rown nécrosis , (b) light nécrosis of soft consistency in sectorial position ,(c) brown hard necrosis around light soft part , (d) complex necrosis (all form) .

Fungal isolation

The classical procedure of isolation was carried out on (1020) samples originating from mature and young vines. Several micro-organisms, pathogens or saprobes, were isolated per wood sample. The main pathogenic wood fungi generally associated with BDA and other trunk diseases were *Botryosphaeria spp*. and *F. mediterranea*. In addition to fungal agent searched, we found a development of fungal flora accompanied by bacterial flora. Among the fungal infection, most frequently encountered genera include *Alternaria, Fusarium, Pestolozzia, Botrytis* and *Penicillium*. This microflora is considered an accompanying flora associated with isolations on vines. This fungal flora was often cited as inhibiting growth of the pathogen concerned.

Comparing distribution of two genus of fungal pathogens according to kind of necrosis (position,texture and discoloration) showed that the higher percentage of fungi was isolated from central or/and sectorial necrosis into the grapevine was attributed to Botryosphaeriaceae whichin *cluded*: *Diplodia* species, *B. dothidea*. *B.viticola* and *N.parvum*. From the central and mixed necrosis, discoloured wood (white) the fungi most frequently isolated was *Fomitiporia mediterranea* .

Morphological and cultural characterization

The colonies of *Botryosphaeria* on PDA media that occur in the first days of incubation were white color and with a cottony texture; some cultures developed to gray pigment that turns to dark green and to black as shown. All isolates of *Botryosphaeria* produced high density and aerial hyphae. Most isolates obtained were identified as *Diplodia* sp. and *Botryosphaeria dothidae*. Some colonies developed, after 3 months,fruiting structures blackish and globular, grouped or single, with pycnidia on the surface. The colonies of second genus was having white color, cottony texture and slow-growing an intense aerial mycelium of the developed hyphae on PDA. With age, some cultures of *Fomitiporia* changed its color, turning from white to yellow which became yellow-brown over time.

Discussion

In Algeria, grapevine declined significantly in recent years because of the deteriorating environmental condition, this disease can be a limiting factor for local vineyards, and may compromise the Algerian wine industry for long period. According to

(Galet,1999) the incidence dieback depends largely on the local climate as perithecia are formed only in the areas where annual rainfall exceeds 300 mm over the rain and wind play a crucial role in the dissemination of ascospores. Our areas of study are located in a bioclimatic or greater than 600 mm rainfall and snow in the region lead to the large spread of diseased wood. Among other factors that may intervene in this process was the vine variety (Dubos,1987), the number, the time, the size of the pruning wounds(Galet,1999), the mode of conduct and the rootstock (Ferreira,1986). In general, the vines in goblets or Royat cordon were not touched as the vines in Guyot, where the annual suppression of arm causes significant the pruning wounds (Galet, 1999). The variability in the incidence of dieback was not a fatality, because of cultural practices, it would be possible to limit these effects, and themost precise of all the factors that may influence disease analysis could provide alternative solutions. However, these results do not enable us to get a clear idea about the magnitude of decline and unavailability of resources to measure the impact from the presence of symptoms, herbaceous organs induce an underestimation of the impact actual disease. The examination of cross and longitudinal sections of trunks collected in vinyards suggested that the presence of many processes of wood colonization causing the degradation and the onset of necrosis, the presence of sectorial hard indicates the presence of the causative agent of eutyposis. According to Dubos (2002), sectorial necrosis is characteristic of eutypiose, the second result of the installation process of the causal agents of esca necrosis causing a light and soft central sectorial position (Mungai *et al*, 1996). The presence of brown band and sis sectoral necrosis brown-gray charctériqtiques aresymptomes of BDA (Larignon, 2010). We can conclude the presence of this kind of nécrosis indicates the presence of two diseases decline in our vineyards (esca and BDA), although on the herbaceous part in our prospecting we have noted just the presence of characteristic symptoms of eutypiosis. This study shows the presence of many trunk pathogens associated with kinds of wood decay symptoms on grapevine in Algeria during the period between 2006 and 2012. The result of the isolations obtained was classified on clusters based on their appearance in agar cultures. Morphological and microscopic characteristics were compared with those reported previously by Fischer (2002) and (Phillips, 2002 ;Úrbez-Torres *et al*, 2006a, 2006b). Four fungal species that causes diseases in the vines were identified *B. dothidae, B.viticola, D. seriata and N. parvum*. All these species of *Botryosphaeriaceae* were isolated from central or/and sectorial necrosis into the grapevine wood. Generally from white and brown lesions confirming that the fungi can be largely found. According to Mugnai *et al*. (1999); Marinelli *et al*. (2012) and Marques *et al* (2013), the *Botryosphaeria* genus was more present in necrotic lines in the current study different from other studies by Larignon and Dubos (1997). According to Savocchia *et al*,(2007) Enarrow black streaks develop in the xylem of infected wood, longitudinal expansion of streaks is most rapid and cross-sections affected trunks and cordons often reveal a wedge ofnecrotic tissue (easily confused with *Eutypa* dieback). The black tissue and bark over the iseased xylem stops to function and dies. From the infection site at the pruning wound, the pathogen moves toward the ground, growing invascular elements as well as in adjacent cells. *F.* mediteranea, the causal agent of esca dieback, was also isolated in this study, low frequencies isolation from wood chips, generally from white and central necrosis. This specie was isolated from grapevine and knowed as grapevine pathogens in different regions of the world. *F.mediterranea* was the causal agent among all other majors pathogenic taxa associated with esca: *Eutypa lata*, *Phaeomoniella chlamydospora,* and *Phaeoacremonium* (Larignon and Dubos, 1997; Mugnai *et al*, 1999; Surico *et al*, 2006). According to Fisher (2002) this species was described as a new wood decaying Basidiomycétes species associated with esca of grape vine in Europrean vine growing countries in southern Europe. *F.mediterranea* does not occur on *Vitis vinfera* but also on a number of other hard wood genera. The epidemiological survey in this study, combined with laboratory analyses

indicates that the observed dieback was complex and it has diverse backgrounds. While it was certain that the two diseases, particularly BDA was involved in the dieback observed, it is also clear that other factors, including abiotic have a direct impact on this disease. To better elucidate this syndrome, it is necessary to involve in other epidemiological studies , all possible factors that directly or indirectly influence the decline syndrome, such as vines, rootstocks, modes of conduct, age as well as weather conditions and understand them. In such a situation, to solve this problem and to draw conclusions about the role of fungi isolated, it is necessary to resort to other methods of complementary diagnostics that can bring new opportunities for faster and more reliable diagnosis.

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