New Ascomycetes Associated with Grapevine Dieback in Algeria

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ABSTRACT

This study was conducted during spring 2012 to detect the causal organism (s) responsible for a new grapevine dieback disease in Algeria. Samples of grapevine wood were collected from 10 grapevine fields located in two regions (Medea and Tipaza). Several fungi were isolated from the margin between healthy and diseased tissues. *Botryosphaeria* spp, were identified based on the morphological characteristics of the culture and confirmed by Beta tubulin (ß-tubulin) region. The sequences submitted to the GenBank (NCBI) under accession numbers *(KC960991)(HQ660477)(AY236931),* revealed 99-100% homology. Other fungal species *Entoleuca mammata* and *Rosellinia merrilli.* were also isolated at low frequency**.** Inoculation *In vitro* of grapevine plantlets, with the two *Botryosphaeriaceae* species, produced smallest necrosis after five-week incubation; *Botryosphaeria obtsusa* (*Diplodia seriata*) were virulent compared with *B. dothidea*. The species tested were re-isolated from necrosis symptoms on infected plantlets .

Keywords: Algeria, grapevine dieback, Phylogenetic analysis, Pathogenicity test.

INTRODUCTION

Black dead arm (BDA) is a frequent trunk disease of grapevine occurring in vineyards all over the world that leads to a slow decline and the death of the plant. However, it is the cause of fatal decline in vine producing countries. This disease is caused by several species of Botryosphaeriaceae, the most frequent being *B. dothidea*, *Diplodia seriata* and *Lasiodiplodia theobromae* (Larignon *et al.*, 2001; van Niekerk *et al.*, 2006). The fungus infects vines through pruning wounds, colonized wood tissues and causes V-shaped necrosis, similar to those caused by *Eutypa. lata*, and longitudinal brown streakings along the affected tissues (Castillo-Pando *et al.*, 2001; Taylor *et al.*, 2005). BDA foliar symptoms reported by Larignon and Dubos (2001), include an early red or yellow-orange patchy discoloration of the leaves (in red- and white-berried grape varieties, respectively) that develop later into large marginal and interveinal necrosis. However, a large similarity between foliar symptoms is shown between these late as BDA (Lecomte *et al*. (2005) and Surico *et al*. (2006). Additionally, some species in the Botryosphaeriaceae have been recognized as opportunistic human pathogens causing subcutaneous, ocular and/or internal organ infections (Woo *et al*., 2008).

A study carried out by Ammad (2014) indicated the existence of several pathogens associated with dieback of vines in Algeria, *Eutypa lata* (Moller, 1978) and *Fomitipria mediterranea* (Dubos,2002), its lignivorous activity causes different kinds of sectorial and central necrosis, hard and soft texture either brown or white in

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Received on 7/4/2014 and Accepted for Publication on 29/5/2014.

color, typical symptoms of two diseases Eutpiosis and Esca respectively. Recently a new symptom was noted on the herbaceous part and in the wood of the grape vine in Algerian vineyards, showing typical symptoms similar to those caused by the balck dead arm (BDA). In view to detect the organism responsible of this third disease called BDA, this study was carried out in ten vineyards located in two regions known by their viticultural vocation. And aimed at identifying and characterizing the causal organisms of grapevine dieback in Algerian vineyards based on morphological characteristics of culture; and, identification was confirmed by analyzing the DNA sequences from selected regions: the (ß-tubulin) .

2. MATERIALS AND METHODS

2.1. Sampling and Isolation

A field survey was conducted in some vineyards in which vines showed dieback symptoms on local cultivars, namely Dattier de Beyrouth, Muscat, Cinsault, Cabernet sauvignon in the northern region of Algeria (Tipaza and Medea) during the spring period of 2012 (Table 1). After carrying out the descriptive symptomatology and localization of the vines with dieback symptoms, samples were collected randomly from each vineyard vines (10 among the 500 observed). Some vines showing symptoms of decline on herbaceous parts of each cultivar were cut at the base of the trunk, wood slices of (<1 mm) were sectioned from the margin between healthy and diseased tissues (Figure.1.a,1.b). Slices were surface disinfected by immersion in sodium hypochlorite (NaOCl) (2%) for 4 min, they were then rinsed with sterilized water and dried in sterilized filter paper. Then, they were placed on Petri plates containing potato dextrose agar (PDA) and incubated at 25°C. Observations of fungal development were recorded weekly. Morphological and microscopic characteristics as (color, form and texture of colonies and form of conidia) of the isolates were in agreement with the description for *B. obtusa* (Johnson,1992) and B. *dothidea* (Qiu *et al*. 2008).

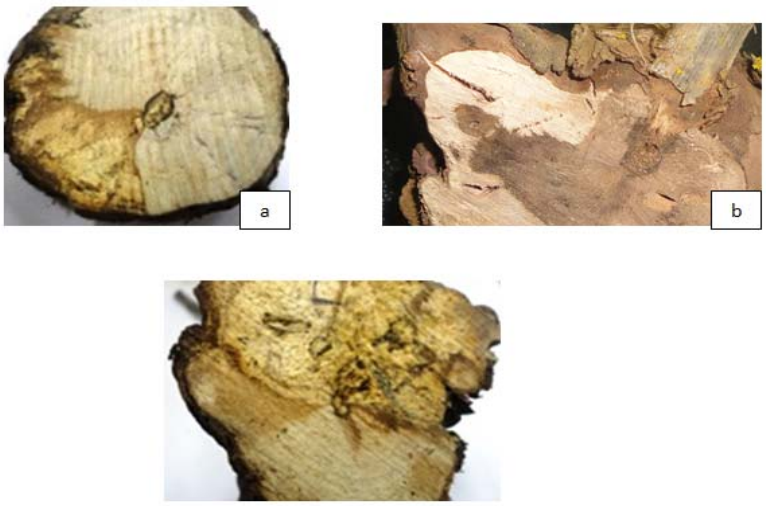

Figure 1: Different form of necrosis: Cross sections and longitudinal sections of trunks associated with typical dieback symptoms on herbaceous parts: (a) sectorial hard brown necrosis, (b) central necrosis position, (c) complex necrosis (all form) .

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

Locality	**Médéa**		**Tipaza**				
site	Benchicao		Hamr El Ain				
Vine yards	1	2	1	2	3	4	5
Cultivar	Dattier de Beyrouth	Carignan	Dattier	Gros noir	Cinsault	Syrah	Cabernet sauvignon
Age (year)	26	45	40	10	12	11	11
Area (Ha)	12	06	05	04	03	04.5	03
Type of Pruning	Guyot simple	Guyot double	Cordon double	Cordon double	Guyot	Guyot	Guyot
Rootstock	41B	41B	41B	41B	SO4	99R	41B

2.2. DNA Extraction and PCR Amplification

Total genomic DNA of all isolates obtained in this study and identified morphologically as Botryosphaeria species, was extracted from 3- to 4-days-old cultures mycelia as reported by Liu *et al.* (2000). The oligonucleotide primer, β-tubulin (5' GGT AAC CAA ATC GGT GCT GCT TTC 3') was used to amplify portion of the β-tubulin as reported by Glass and Donaldson (1995). The amplification reactions were performed in a 25µl volume of reaction mixture containing (1mM of each primer, 0.2 Mm of dNTP, 15 ml $MgCL_2$ and 2.5 U of Taq polymerase adjusted with purified distilled water to a final volume of 25 µl). The PCR program which was run according to Guizhen and Mitchell (2002) included an initial denaturation at 95 C° for 2 min, followed by 35 cycles of 1 min denaturation at 94 C°, annealing for 40 s at 53 C°, and 1 min elongation at 72 C°, with final elongation step at 72 C° for 10 min. The PCR products were separated by agarose gel electrophoresis (100 V for 60 min) in 1.5% agarose gels prepared in TBE buffer 0,5 X (Tris-Borate 100 Mm; pH 8,3; EDTA 2 mM) added 50 µg ethidium bromide (BET), and visualized under UV light (Sambrook *et al*., 1989). The PCR products were purified with QIA quick Wizard PCR purification Kit (Promega) according to the manufacturer's instructions. The sequences were determined by a cycle sequencing using the Taq Dye Deoxy Terminator Cycle sequencing kit (Applied Biosystems, HTDS, Tunisia).

2.3. Molecular Identification

The nucleotide sequences of six isolates were aligned with the multiple sequence alignment program Chromas1.7.5 (http://www.technelysium.com.au/ chromac. html) All sequences were checked manually and they were initially analyzed by searching the National Center for Biotechnology Information (NCBI) database using the BLAST (Basic local alignment search tool) (Altschul *et al*., 1997), searches and included in the alignment. Phylogenetic analysis also was performed for the β-tubulin dataset. Maximum parsimony analysis was performed with Mega Version 5.02. The analysis consisted of heuristic searches with 1,000 repetitions of random terminal addition of sequences. Reference sequences for the β-tubulin regions for the *Botryosphaeria* species were obtained from GenBank. The β-tubulin sequences were aligned with Clustal X v.1 using pairwise alignment parameters (gap opening = 10 gap extension = 0.1), multiple alignment parameters (gap opening = 10 gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 30%), and manual

adjustments made where necessary.

2.4.Pathogenicity test

Pathogenicity tests were performed *in - vitro* on grapevine -plantlets using Algerian local cultivar: Dattier de Beyrouth that were maintained in *in - vitro* micro-propagation culture. Micro-cuttings (fragment of stem, leaf and a bud) were placed after disinfection on the ML-vitis medium. The micro cuttings were transplanted, under aseptic conditions, in a test tube for inoculation with fungi identified in this study. The *in - vitro*-grown plantlets were inoculated with two isolates belonging to two fungal species isolated in this study: *B. dothidea* and *B. obtusa (D. seriata)*, under controlled condition. Five plantlets per isolate and two isolates per species were used. The mycelial suspension was passed through glass wool to remove hyphal fragment. The filtrate contained spores was collected, and conidia suspension was prepared. Using fine sterile needles; 1mL of each fungal suspension (10^6conidia/mL) was injected into superficial wounds at different positions without touching the cambium of the vines. The inoculation sites were covered by cotton swabs moistened with sterile water to avoid desiccation. Five *in - vitro*-grown plantlets were used as negative controls and were inoculated with sterile distilled water. *In vitro*-gown plantlets were observed after five weeks and inspected for development of disease symptoms. The intensity of infection was evaluated via area of infection and apparition of necrosis from the inoculation point; and was classified as low, medium and high by visual observations. Small fragments (0.5 to 1cm) of necrotic tissue were placed on PDA plates to determine the cause of lesion. After five days, fungi were identified based on cultural and morphological characteristics.

3. RESULTS

3.1. Fungal Isolation

Isolations were attempted from 200 samples with V-shaped necrosis, based on their appearance in culture and the isolates obtained in this study were assigned to Botryosphaeria genus. The isolates obtained, occurred in the first days (2-3 days) of incubation; these later became white in color and of a cottony texture. All isolates of *Botryosphaeria* genus produced high density aerial hyphae. After 10 days of incubation, some isolates developed grey mycelia that turn to dark green and black. Among the isolates obtained, 25 % of isolates conformed to the *Diplodia* anamorph of the genus *Botryosphaeria* and were identified as *B. obtusa*. The conidia from these isolates were hyaline when immature and dark brown at maturity, mostly aseptate but occasionally with 1-septate (Figure.2.a). The remaining isolates were identified as *Botryosphaeria dothidea*. Colonies were initially white after 3 days of incubation, becoming grey or dark grey by age, also with abundant aerial mycelium Conidia were fusiform to fusiform - ellipsoid with a truncate base and sub-obtuse apex (Figure.2.b and 2.c), aseptate, hyaline, thin-walled. The *B. obtusa* and *B. dothidea* were present in the two regions surveyed (Table.2), and isolated with different frequencies from all cultivar of grapevine sampled. Other fungi such as *Entoleuca mammata* and *Rosellinia merrillii*.were isolated from grapevine cankers, Six isolates were selected for molecular analysis.

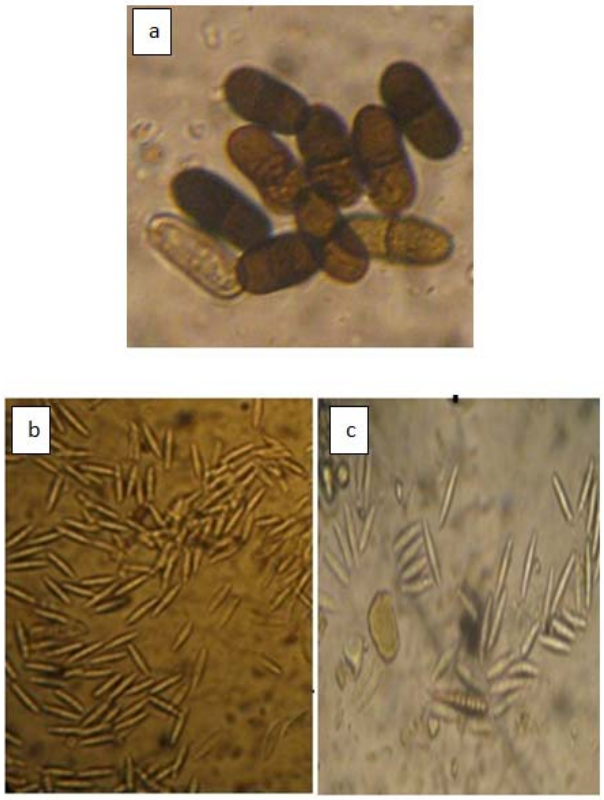

Figure 2:(a) mature, dark septate conidia *D. seriata*; (b)and (c) conidia of *B. dothidea,* Scale bar = µm10

3.2. Molecular Identification

Sequence alignments *for Botryosphaeria* isolates was done by using BLAST in GenBank, which showed that 75% of sequences were having 99–100% homology with *B. dothidea* and the remainig (25%) showed the similar frequency with *Diplodia seriata.*

Phylogenetic analysis was performed only with genera of *Botryosphaeriaceae* species, using maximum composite likelihood for the construction of maximum parsimony.

The β-tubulin dataset comprised sequences from seven isolates collected in this study, and 22 reference sequences retrieved from GenBank, allowed the identification of two groups. The alignment contained 317 characters including coded alignment gaps. After a heuristic search, the maximum parsimony analysis of the β-tubulin region (CI = 0.96, RI = 0.99, HI = 0.05). The isolates from Algeria were distributed over two clades: the first corresponded to the genus *Botryosphaeria obtusa (Diplodia seriata)* with 100% bootstrap support, and the second group was composed of *Botryosphaeria dothidea*.(Figure 3)

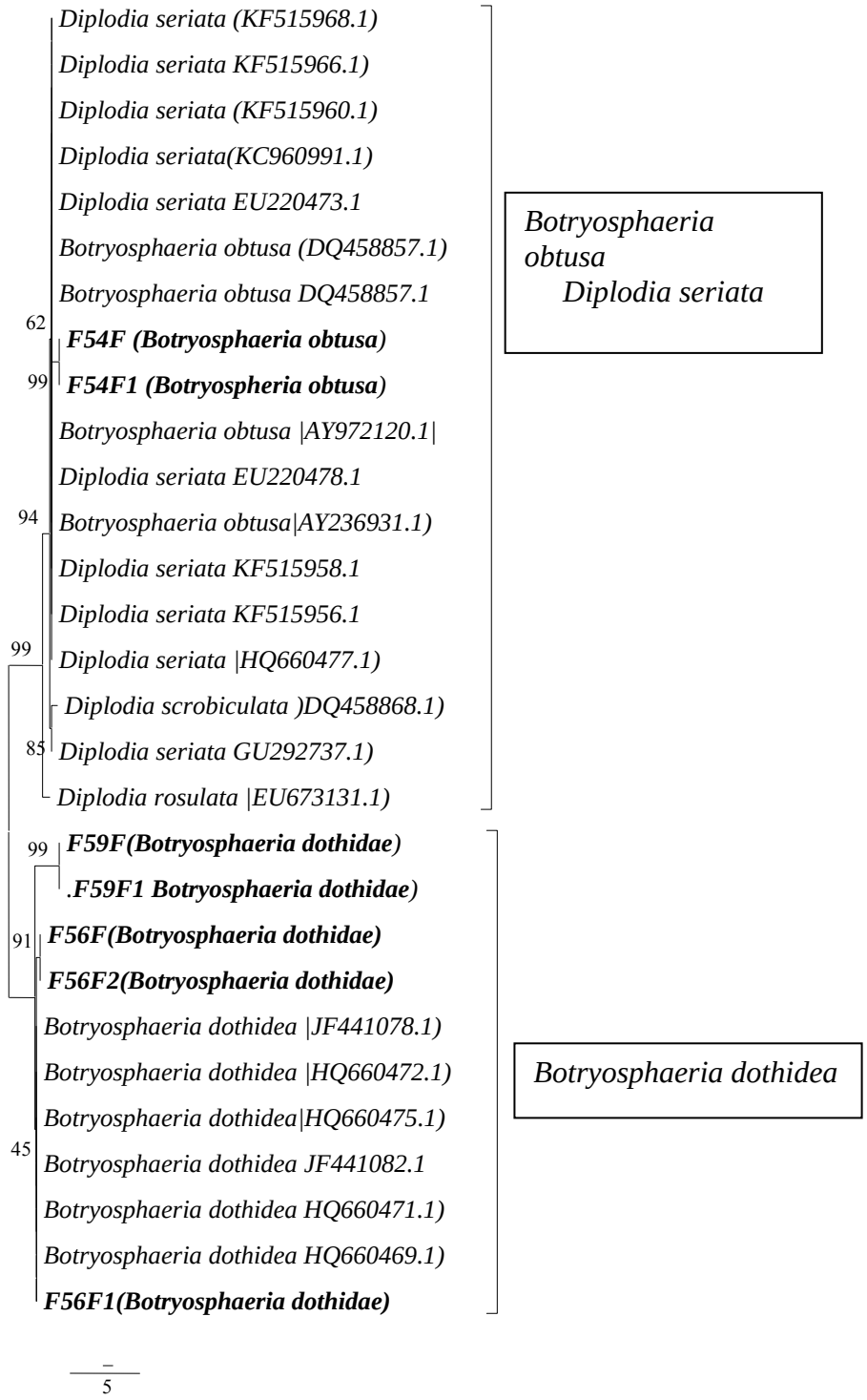

Figure 3. One of five equally parsimonious trees resulting from the alignment. Bootstrap values from 1000 replications are shown for Maximum parsimony (MP).

3.3.Pathogenicity test

Diplodia seriata and *B. dothidea*, isolates inoculated on grapevine plantlets of Dattier de Beyrouth cultivar, showed upward and downward from necrosis at the point of inoculation, and were significantly different from the control. These isolates caused the smallest necrosis. For the 40 infected *in - vitro*-grown plantlets, the following distribution was observed when scoring symptoms: 15 were without symptoms, and 25 vitro-plantlets with symptoms (Figure.4). The tested species were re-isolated from the margins of necrosis and their identity confirmed on the basis of morphological and cultural characters. No fungi were isolated from the controls.

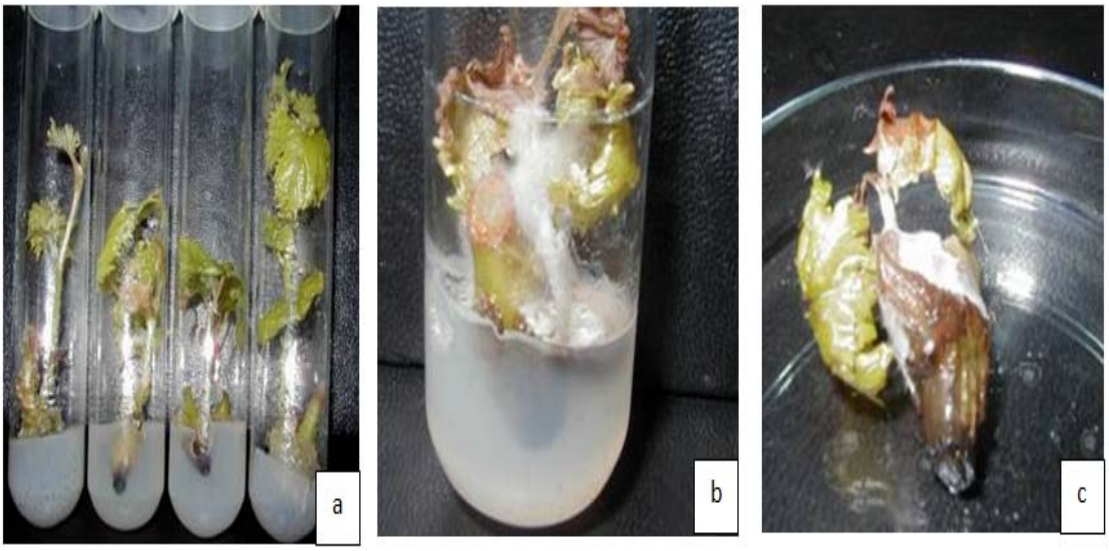

Figure 4 : After five weks of inoculation (a) vitro plantlet infected, (b) and (c) mycelial filaments *in vitro*

4. DISCUSSION

The present study revealed the presence of a fungal diversity associated with wood cankers and grapevine dieback in Algeria. Grapevine dieback have been known to occur in Algeria since 2006, when *Eutypa* dieback and Esca were associated with grapevine decline in some vineyards in the grape-growing areas . Given that *E. lata* has been considered as the primary canker causing agent of grapes worldwide. The result of isolates characterization based on morphological characteristics combined with analysis of DNA sequences allowed us to identify *Botryosphaeria* species accompanied with other genus. Isolates obtained were classified in two clusters based on their appearance in culture and conidial morphology. Morphological and microscopic characters are compared with those reported previously by Phillips (2002). Those species that were isolated from grapevine in Algeria are known as grapevine pathogens in different regions of the world. Several studies confirmed that Botryosphaeria sepecies were the causal organism (s) of black dead arm dieback, an important perennial canker disease that occurred in most countries where grapevine is cultivated (Larignon *et al.*, 2001). The association between those fungi and *Vitis vinifera* was of particular relevance, since several species of this family are significant plant pathogens causing leaf spots, fruit rots,

dieback, perennial cankers, wood streaking, wedge-shaped discoloration in wood, shoot dieback and cane bleaching in all major viticulture regions throughout the world and eventual death in economically important woody perennial crops (Van Niekerk *et al*.2006; Úrbez-Torres *et al*. 2009). The identity of species was further confirmed by amplification and sequencing of the rDNA, β-tubulin gene and unique morphological characters. The present work has allowed the analysis of the fungi isolated from necrotic wood revealed the existence of *Botryosphaeria* genus involved in the decline. *Diplodia seriata* was predominantly isolated from the V-shaped necrosis, with 25% of the isolations made from arms and trunks. Luque *et al*.,(2009) reported similar results . However, the remaining species of Botryosphaeriaceae (*B. dothidea*) accounted for an additional 75 % of isolations from arms and trunks. These species have a wide host range, many studies have been reported Botryosphaeriaceae species causing the same symptomatology on perennial woody crops such as apple, peach, olives and pistachio (Sutton, 1981; Michailides, 1991; Copes and Hendrix, 2004), and represent well-known grapevine pathogens all over the world as in Spain (Aroca *et al*., 2006), France (Larignon *et al*., 2001). Pathogenicity studies conducted *in vitro* in this work, , were carried out with scoring symptoms, and re-isolation of fungi, revealed the presence of *D. seriata* and *B. dothidea* that caused the smallest necrosis on Dattier de Beyrouth grapevine. *D. seriata* virulence was estimated by how early the symptoms appeared compared with the second specie *B. dothidae*. Bertsch et *al*.(,2013) reported that *In vitro* cultures are excellent tools for studying host–pathogen-interactions, as the organisms are grown well in controlled conditions. Co-culturing grapevine calli with the responsible agent of esca has been shown to reduce callus growth, increase plant cell lipid peroxidation and induce browning and necrosis (Sparapano et *al*., 2000c, 2001a). Regarding the pathogenic fungi involved in botryosphaeria dieback, some discoloration of woody tissues and canker formations are commonly observed in cuttings, detached woody shoots or field-grown grapevine shoots that have been inoculated with *D. seriata* (Castillo-Pando et *al*., 2001. Larignon et *al*., 2001; Savochina et *al*, 2007). *Botryosphaeria obtusa* is a fungal pathogen associated with black dead arm (BDA) on vine. It was reported as a pathogen of grape vine in Chili (Auger, 2004). Van Niekerk *et al*. (2004) concluded that this species produces an important larger lesions in canes of vine, Different studies have reported *D. seriata* to be associated with grapevine decline symptoms such as trunk and bark infections in Herzegovina, Yugoslavia (Radman and Nadazdin, 1981), xylem necrosis in Italy (Rovesti and Montermini, 1987), perennial cankers in Spain (Úrbez-Torres *et al*., 2006), Black dead arm in Lebanon (Choueiri *et al*., 2006). Therefore, whether *D. seriata* is acting as a saprophyte or is a pathogen causing grapevine dieback symptoms has not yet been clarified in many grape-growing regions worldwide. However Taylor *et al*. (2005) reported *B. obtusa* likely to be saprophyte. This study also represents the record of *B. dothidea* on grape vine. It has a world-wide distribution and is capable of infecting numerous plant species. Its host range comprises mostly trees and shrubs and even 70 years ago it was reported from 68 genera (Smith, 1934). In Spain, this species is considered as the most common species associated with grapevine (*Vitis vinifera*) decline syndrome (Aroca *et al.* 2006). It was confirmed to be associated with band canker of almond trees in California (Inderbitzin *et al.*, 2010). Recent analysis has confirmed the presence of *B. dothidea*, along with other *Botryosphaeria* species, on grapevine in Tunisia (Chebli et *al*.,2014). While it is best known as a pathogen, the species has also been identified as an

endophyte, existing in association with plant tissues on which disease symptoms were not observed (Pérez *et al*., 2010). It can colonize some fruits, in addition to woody tissues (Lazzizera *et al*.2008 and Marques *et al*., 2013).

To our knowledge, this is the first report on the genus *Botryosphaeria* being isolated from grape region in Algeria. Further work will be also needed to improve the control of these pathogens, and to ascertain further the role of environmental and cultural factors that may favor their development under local conditions.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Mr C. Ameur (University of Manouba, Tunis) Dr I. Sbissi (University d'El Manar, Tunis) and Dr N.Belkacem (University d'El Manar, Tunis)for their technical assistance. We also extend our thanks to Ms. H. Makeni (University of Tunis), Pr A. Zitouni (ENS Kouba, Algeria) and S. Amrine (University Blida1, Algeria) for their relevant recommendations.

REFERENCES

Ammad, F.; Benchabane, M.;Toumi, M. 2014. Diversity of Fungal Trunk Pathogens Associated with Grapevine Dieback of Grapevine in Algeria .***Jordan journal of Biological Sciences***, 7: 35-39.

Altschul, S. F.; Madden, T. L.; Schaffer, A.A.; Zhang, J. H.; Zhang, Z.; Miller W. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. ***Nucleic Acids Res***., 25: 3389–3402.

Aroca, A.; Garcia-Figueres, F.; Bracamonte, L.; Luque, J.; Raposo, R. 2006. A survey of trunk disease pathogens within rootstocks of grapevines in Spain. ***European Journal of Plant Pathology***, 115: 195–202.

Auger, J., Esterio M., Ricke G., Pérez I., 2004. Black dead arm and basal canker of *Vitis vinifera* cv. Red globe caused by *Botryosphaeria obtusa* in Chile. ***Plant Disease***, 88: 1286.

Bertsch, A., Ramı´rez-Suero, M., Magnin-Robert, M, Larignon, P., Chong, L ., Abou-Mansou,E., Spagnolo,A., Cle´menC., and Fontaine,F. 2013. Grapevine trunk diseases: complex and still poorly understoodt. ***Plant Pathology***, 62 (2)88: 243-265.

Castillo-Pando, M., Somers A., Green C.D., Priest M.,Sriskanthades M., 2001. Fungi associated with dieback of Semillon grapevines in the Hunter Valley of New South Wales. ***Australasian Plant Pathology***, 30: 59-63.

Chebil, S.; Fersi, R.; Yakoub, A. and S. Chenenaoui, S, 2014. First Report of *Botryosphaeria dothidea, Diplodia seriata*, and *Neofusicoccum luteum* Associated with Canker and Dieback of Grapevines in Tunisia, Disease Notes. ***Plant Disease***, 98: (3): 420

Choueiri, E., Jreijiri, F., Chlela, P., Louvet,G., Lecomte, P., 2006, Occurrence of grapevine declines and first report of Black dead arm associated with *Botryosphaeria obtusa* in Lebanon. ***Plant Disease***, 90: 115.

Copes, W. E.; Hendrix, F. F. Jr., 2004. Effect of temperature on sporulation of *Botryosphaeria dothidea*, *B. obtusa*, and *B. rhodina*. ***Plant Disease***, 88 : 292–296.

Dubos, B., 2002. ***Maladies cryptogamiques de la vigne***. 207p. Bordeaux, Editions Féret.

Glass, N. L and Donaldson, G. C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. ***Appl. Environ. Microbiol***., 61: 1323–1330.

Guizhen, L. and Mitchell**,** T. G. 2002. Rapid identification of pathogenic fungi directly from cultures by using multiplex PCR. ***Journal of Clinical Microbiology***, 40 (8): 2860–2865.

Johnson, G. I. 1992. Biology and control of stem end rot pathogens of mango. Ph.D Dissertation, University of Queensland. Australia.

Larignon, P.; Fulchic, R.; Laurent, C.; B. Dubos, B., 2001. Observation on black dead arm in French vineyards. ***Phytopathologia Mediterranea***, 40: S336–S342.

Lazzizera, C.; Frisullo, S.; Alves, A.; Phillips, A. J. L. 2008. Morphology, phylogeny and pathogenicity of *Botryosphaeria* and *Neofusicoccum* species associated with drupe rot of olives in southern Italy. ***Plant Pathology***. 57:948-956.

Lecomte, P.; Leyo, M.; Louvet, G.; Corio-Costet, M.F. ; Gaudillère, J. P.; Blancard, D. 2005. Le Black dead arm, genèse des symptoms. ***Phytoma***, 587: 29–37.

Lehoczky, J. 1974. Black Dead-Arm disease of grapevines caused by *Botryosphaeria stevensii* infection. ***Acta Phytopathologica Academiae Scientiarum Hungaricae***, 9: 319–327.

Liu, D., Coloe, S., Baird, S., Pederson J., 2000. Rapid mini preparation of fungal DNA for ***PCR***. ***J. Clin. Microbiol***., 38(1): 471.

Marques, M. W., Lima, N. B.; M. A. de Morais Jr.; Michereff, S. J; Phillips A. J. L. ;Câmara, P. S. 2013. *Botryosphaeria*, *Neofusicoccum*, *Neoscytalidium* and *Pseudofusicoccum* species associated with mango in Brazil. ***Fungal Diversity***, 61: 195–208.

Michailides, T. J. 1991. Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. ***Phytopathology***, 81: 566–573.

Moller, M. J ; Kasimatis N. (1978). Dieback of grapevines caused by *Eutypa armeniacae*. ***Plant Disease Report***, 62: 254-258.

Pérez, C.A.; Wingfield, M. J; Slippers, B.; Altier, N. A; Blanchette, R.A. 2010. Endophytic and canker-associated *Botryosphaeriaceae* occurring on non-native *Eucalyptus* and native *Myrtaceae* trees in Uruguay. ***Fungal Diversity***, 41: 53-69.

Phillips, A. J. L. 2002. *Botryosphaeria* species associated with diseases of grapevines in Portugal. ***Phytopathologia Mediterranea***, 41: 3–18.

Qiu, Y.; Savocchia, S.; Steel, C. C.; Ash G. J. 2008 *Botryosphaeria dothidea* associated with grapevine trunk disease in south-eastern Australia. Australas. ***Plant. Pathol***., 37: 482–485.

Radman, L. and Nadazdin, V. 1981. A contribution to the study of two *Sphaeropsis* species parasites of the bark of grapevine in Herzegovina,Yugoslavia. ***Phytopathol. Mediterr***., 20: 83-84.

Romanazzi, G.; Murolo, S.; Pizzichini, L.; Nardi, S. 2009. Esca in young and mature vineyards and molecular diagnosis of the associated fungi. ***Eur. J. Plant. Pathol***., 125: 277-290.

Rovesti, L.; Montermini, A.. 1987. Un deperimento dellavite causato da *Sphaeropsis malorum* diffuso in provincial di Reggio Emilia. ***Informatore Fitopatologico***, 37: 59–61

Sambrook, J.; Fritsh, E. F.; Maniatis, T. 1989. Molecular Cloning : A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory.

Savocchia, S.; Steel, C.C.; Stodard, B.J.; Somers, A. 2007. Pathogenicity of Botryosphaeria species isolated from declining grapevines in sub tropical regions of Eastern Australia. Vitis ,46: 27–32.

Slippers, B.; Smit, W. A.; Crous, P. W.; Coutinho, T. A.; Wingfield, B. D.; Wingfield, M. J. 2007. Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruit trees in South Africa and other regions of the world. ***Plant Pathology***, 56: 128 –139.

Smith, C. O. 1934. Inoculations showing the wide host range of *Botryosphaeria ribis*. ***Journal of Agricultural Research***, 49: 467-476.

Sparapano, L.; Bruno, G. ; Graniti, A. 2000c. Effects on plant of metabolites produced in culture by *Phaeoacremonium chlamydosporum*,*P. aleophilumand Fomitiporia punctata*.***Phytopathologia Mediterranea***, 39: 169–77.

Sparapano, L.; De Leonardis, S.; Campanella, A.; Bruno, G. 2001a. Interaction between esca-associated fungi, grapevine calli and micropropagated shoot cultures of grapevine.Ph***ytopathologiaMediterranea***, 40:S423–8.

Surico, G.; Mugnai, L.; Marchi, G. 2006. Older and more recent observations on Esca: a critical overview. ***Phytopathologia Mediterranea***, 45: 68-86.

Sutton, T.B. 1981. Production and dispersal of ascospores and conidia by *Physalospora obtusa* and *Botryosphaeria dothidea* in apple orchards. ***Phytopathology***, 71: 584–589.

Taylor, A.; Hardy, G. E. St.J.; Wood, P.; Burgess, T. 2005. Identification and pathogenicity of *Botryosphaeria* species associated with grapevine decline in Western Australia. ***Australasian Plant Pathology***. 34: 187-195

Úrbez-Torres, J. R.; Peláez, H.; Santiago, Y.,; Martín, C.; Moreno, C.; Gubler, W.D. 2006. Occurrence of *Botryosphaeria obtusa*, *B. dothidea* and *B. parva* associated with grapevine trunk diseases in Castilla y León region, Spain. ***Plant Disease***, 90,: 83

Úrbez-Torres, J.R., Gubler, W. D. 2009. Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California. ***Plant Disease***, 93: 584–592.

Van Niekerk, J. M.; Crous, P.W.; Groenewald, J. Z.; Fourie, P.H.; Halleen, F. 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines Mycologia, 96: 781–798.

Van Niekerk, J. M.; Fourie, P. H.; Halleen, F.; Crous, P.W.,2006. *Botryosphaeria* spp. as grapevine trunk disease pathogens. ***Phytopathologia Mediterranea***, 45: 43-54.

White, T. J.; Bruns,T.; Lee, S.;Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Snisky JJ and White TJ (Eds), PCR Protocols: A Guide To Methods and Applications. San Diego : Academic Press, pages, 315–322.

Woo, P. C. Y.; Lau, S. K. P.; Ngan, A. H. Y. H. Tse, Tung, E. T. K.; Yuen, K. Y. ,2008. *Lasiodiplodia theobromae pneumonia* in a liver transplant recipient. ***Journal of Clinical Microbology***,46: 380–384..

أنواع فطر زقي جديدة مرتبطة بالموت التراجعي لكرمة العنب في الجزائر

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ملخص

أجريت هذه الدراسة خلال فصل ربيع عام 2012 للكشف عن الفطور المسؤولة عن مرض الموت التراجعي (السقم) في الجزائر. تم جمع عينات من خشب كرمة العنب في 10 كروم عنب في منطقتين (ولاية تيبازة والمدية). تم عزل العديد من الفطور من بين الحافة الفاصلة بين الأنسجة السليمة والمريضة للخشب. تم التعرف إلى الفطور التابعة لأنواع : *Botryosphaeria* spp. على أساس الخصائص المورفولوجية وتم تأكيد التعريف باستعمال فحص تفاعل البلمرة المتسلسل الموزثة ß-tubulin كشفت التسلسلات *(KC960991)(HQ660477)(AY236931)*المودعة في بنك المورثات (NCBI)عن نسبة تماثل 99 –100٪. تم أيضا عزل فطور من أنواع أخرى *Entoleuca mammata* و *Rosellinia merrilli* بنسبة ضعيفة. التلقيح شتلات العنب، بنوعين من Botryosphaeriaceae ، أدى إلى ظهور نخر صغيرة بعدة خمسة أسابيع امن التلقيح ؛ *Botryosphaeria obtsusa* (*Diplodia seriata*)ابدت شراسة مقارنة مع *B. dothidea* . . تم عزل أنواع الفطور المستعملة من أعراض نخر على الشتلات الملقحة .

الكلمات الدالة: الجزائر. الأمراض التي تصيب جذع كرمة العنب، تحليل فيلوجيني– القدرة الإمراضية.

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تاريخ استلام البحث 2014/4/7 وتاريخ قبوله 2014/5/29.