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Phaeoacremonium species associated with Eutypa dieback and esca of grapevines in Algeria

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Summary. Algerian grapevines showing symptoms of Eutypa dieback and esca were examined for the presence of *Phaeoacremonium* species. Species were identified on the basis of morphological and cultural characteristics as well as DNA sequence data (β-tubulin and actin). From a total of 200 vines sampled, 61 *Phaeoacremonium* isolates were obtained corresponding to four different species. *Pm. aleophilum* was the most frequent (42 isolates), followed by *Pm. parasiticum* (10 isolates) and *Pm. venezuelense* (8 isolates). *Phaeoacremonium hispanicum* was also found but only once. *Phaeoacremonium* species were more frequently associated with Eutypa dieback than with esca symptoms. This correlates with their frequent association with sectorial brown necrosis (V-shaped necrosis).

Key words: actin, β-tubulin, phylogeny, wood disease.

Introduction

Algeria is one of the oldest wine- producing countries in the world and viticulture began well before the time of the Roman Empire. The increase of grape production in Algeria at the end of the 19th century was due to the phylloxera epidemic that affected European vineyards and also to the favourable soil and climate of the country. By 1938, the cultivated area of grapevines had reached a peak of 400,000 ha producing 22 million hectolitres of wine (Hildebert, 1949). Nowadays, viticulture still occupies an important place in Algerian agriculture. According to statistics from the Ministry of Agriculture for 2009 (Anonymous, 2009), grapevines are planted on 82,743 ha producing 492,525 tons of grapes for table, wine and raisins.

Diseases such as powdery mildew, downy mildew, black-rot and excoriose are common throughout wine growing areas and cause heavy economic losses. Trunk diseases of grapevine are also very harmful, and affect the productivity and the longevity of vineyards. Trunk diseases are characterized by a slow decline leading to the death of the vines. Debraye (1892) reported in Algeria cases of declining vines that he called "apoplexy". Ravaz (1905) also reported high mortality rates in many viticulture areas of Algeria. He suggested that numerous factors were involved, such as the vigor of the vines and a climate that is conducive to such damage. Since then, there were no other studies until 2003 when a preliminary survey was undertaken in several regions. That survey revealed a

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high percentage of dead vines in some vineyards and the occurence of both Eutypa dieback and esca (Berraf and Péros, 2005). The survey showed that Eutypa dieback was more common in vineyards than esca, with 37% of vines affected by Eutypa dieback and 15% with esca. Berraf and Péros (2005) also noted that the dying arms symptom was mainly a result of Eutypa dieback.

Symptoms of Eutypa dieback and esca are well-characterized, appearing in early spring as stunted shoots with small, chlorotic, cup-shaped lesions with a necrotic margin. Cross-sections of arms and trunks of infected vines show wedge-shaped discoloured sectors (Moller and Kasimatis, 1978). If the disease progresses, the entire vine may die within 10 years of infection (Pascoe and Cottral, 2000).

Esca is typically identified by internal wood decay, and by the symptoms on the leaves, and in some cases on the berries (Gubler *et al.,* 2004a). The disease can appear in a mild form, characterized by leaf alterations (Mugnai *et al.,* 1999) or in a severe form, characterized by a sudden wilt of the plant often called "apoplexy". Apoplexy is frequent in the Mediterranean area when a hot dry period is preceded by rainfall (Viala, 1926). The internal symptoms of esca include black spots and dark brown to black streaking of the xylem tissues. These symptoms have been reported in grapevines wherever they are grown, with severity increasing year by year (Mugnai *et al.,* 1999). Several studies have shown that a number of fungi are associated with Eutypa dieback (Ferreira *et al.,* 1989; Luque *et al.,* 2009) and also with esca (Larignon and Dubos, 1997; Péros *et al.,* 2008, Luque *et al.,* 2009). The most frequent fungi are *Eutypa lata*, the cause of Eutypa dieback (Carter, 1991), several species of *Phaeoacremonium* (Mostert *et al.,* 2006a; Essakhi *et al.,* 2008; Gramaje *et al.,* 2009), *Phaeomoniella chlamydospora* (Crous and Gams, 2000), several species of Botryosphaeriaceae (Phillips, 2002), and the basidiomycete *Fomitiporia mediterranea* (Fischer, 2002).

The survey carried out by Berraf and Péros (2005) revealed that the fungal community in decaying vines in Algeria was similar to fungal communities in other countries. However, *Phaeoacremonium aleophilum* was found at a higher frequency and these authors suggested that this species could be favoured by the hot Algerian climate. In Australia this species is more frequent in hotter regions (Edwards and Pascoe, 2004), and it is less common in Northern France than in southern France (Larignon, personal communication). Furthermore, in the first survey performed in Algeria, the possibility that other *Phaeoacremonium* species may also infect grapevine was not assessed. Different *Phaeoacremonium* species have indeed been isolated from a wide range of hosts such as humans, woody plants, larvae of bark beetles and soil. These species are opportunistic pathogens needing a subcutaneous traumatic inoculation or a predisposed host to infect, and to cause disease in humans (Ajello *et al.,* 1974; Mostert *et al.,* 2006a). Some species, such as *Pm. krajdenii*, *Pm. parasiticum*, *Pm. venezuelense* and the most common, *Pm. aleophilum* have also been isolated from other woody hosts (Larignon and Dubos, 1997; Mostert *et al.,* 2006a; Essakhi *et al.,* 2008; Gramaje *et al.,* 2009).

The purpose of this study was to identify the *Phaeoacremonium* species associated with Eutypa dieback and esca in Algeria. We examined a large number of decaying vines and *Phaeoacremonium* species were identified based on their morphological characteristics and their DNA sequences. In addition, we studied where the species were located within the vine (trunk or arm) as well as in which type of wood lesion.

Materials and methods

Analysis of internal symptoms and isolation

A total of 200 vines cv. Cinsault planted in 1981, 100 with mild or severe forms of esca and 100 showing symptoms of Eutypa dieback were sampled in the main production areas of the northern Algeria. Cross and longitudinal sections of the trunks and arms of each vine were examined to record the type and location of the wood necrosis. Isolations were made from each type of necrotic tissues. For each lesion detected, 10 pieces of wood (10×5×5 mm) were cut from the margin of the soft white rot, the sectorial and the central brown zone and the black spots as described by Larignon and Dubos (1997) and Luque *et al.* (2009). The pieces of wood were surface disinfected with calcium hypochlorite (3% active chlorine) for 10 min, rinsed twice in sterile water and then placed on potato-dextrose agar (PDA, Difco Labo-

ratories, Detroit, MI, USA) plates. Plates were incubated at room temperature and inspected every 2–3 days for two months. *Phaeoacremonium* species were transferred to fresh PDA plates. A *Phaeoacremonium* species was associated with a lesion type when at least one of the 10 pieces of tissue yielded that species. Morphological characters to distinguish species of *Phaeoacremonium* included conidiophore morphology, phialide type and shape, size of hyphal warts. Colony characters and pigment production were noted after 8 and 16 days of incubation at 25°C on malt extract agar (MEA: 2% malt extract Difco, 1.5% agar), PDA and oatmeal agar (OA) (Gams *et al.,* 2007). Colony colours were defined after 16 d using the colour charts of Rayner (1970).

DNA isolation

Genomic DNA of all isolates identified morphologically as *Phaeoacremonium* was extracted from fresh mycelium grown on PDA plates in darkness at 25°C for 2–3 weeks following Santos and Phillips (2009).

MSP-PCR profiles

The *Phaeoacremonium* isolates were initially characterized on the basis of their microsatellite primed-PCR (MSP-PCR) profiles as described by Alves *et al*. (2004). The primer used for the MSP-PCR was M13 (5'– GAG GGT GGC GGT TCT– 3') (Meyer *et al.,* 1993). The reaction mix in a final volume of 25 µL contained 1×PCR buffer (20 pmol of primer, 200 µm of one of each dNTP, 1.25U of *Taq* DNA polymerase (MBI Fermentas, Vilnius, Lithuania), 3 Mm of $MgCl_2$ and 10 ng of template DNA. The cycling conditions were: 2 min at 94°C, followed by 40 cycles of 30 s at 93°C, 3 s at 53°C and 2 min at 72°C, then a final step of 10 min at 72°C. The amplification products were separated by electrophoresis in 1.5% (w:v) agarose gels in 0.5×TBE (Tris Borate EDTA) for 3 h 30 min at 80 V. Gel electrophoresis images were acquired under UV illumination with the Molecular Imager Gel Doc XR System (Bio-Rad, Hercules, CA, USA), after staining with Gel Red (Biotium, Hayward, CA, USA). DNA banding patterns were analyzed with GELCOMPAR (version 4.1, Applied Maths Kortrijk, Begium, 1998) using Pearson's correlation coefficient and the dendrogram was computed using UPGMA clustering. The reproducibility level was calculated by comparing the banding profiles resulting from independent amplification of 10% of these isolates chosen randomly.

Sequence analysis

Two gene regions were amplified. A fragment of around 600 bp of the β-tubulin (TUB) gene was amplified using the primers T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995), and a fragment of around 300 bp of the actin (ACT) gene was amplified as described by Mostert *et al*. (2006b) using the primers ACT 512F and ACT 783R (Carbone and Kohn, 1999). The reaction mixture contained 50–100 ng of genomic DNA, 15 pmol of each primer, 200 µm of one of each dNTP, 3 mM MgCl2, 1% DMSO to improve the amplification of some DNA templates and 1 U of *Taq* DNA polymerase. Each reaction volume was brought to 50 µL with sterile water. The amplification conditions for TUB regions were: 5 min at 94°C, followed by 40 cycles of 30 s at 94°C, 3 s at 52°C and 1 min at 72°C, and a final step of 10 min at 72°C. PCR products were purified according to the manufacturer's instructions using the Nucleo Spin Extract II commercial kit (Macherey-Nagel, Düren, Germany). The TUB and ACT regions were sequenced by STAB Vida, Lda (Oeiras, Portugal). Newly generated sequences were deposited in GenBank (Table 1).

Sequences for the two DNA regions were retrieved in GenBank (Table 1) using the BLAST (Basic local alignment search tool) (Altschul *et al.* 1990). The sequences of *Pleurostomophora richardsiae* (CBS 270.33; GenBank ACT: AY579271; TUB: AY579334) and *Wuestineaia molokaiensis* (STE-U3797; GenBank ACT: AY579335; TUB: AY579272) were used as outgroups. Sequences were edited with BioEdit Alignment Editor V.7.0.9.0 (Hall, 1999) and aligned with Clustal X version 1.83 (Thompson *et al.,* 1997). Alignments were checked and manual adjustments were made when necessary. Phylogenetic analyses were carried out using PAUP v4.0b10 (Swofford, 2003) for maximum-parsimony (MP) and Neighbour joining (NJ) analyses. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. The trees were visualized with TreeView (Page, 1996).

Table1. Isolation details and GenBank accession numbers of the isolates obtained in the current study and included in the phylogenetic analysis.

Species	Isolate number[a]	Origin	Host	Collector	GenBank accession numbers	
Phaeoacremonium aleophilum (Togninia minima)	STE-U 5836	South Africa	*Prunus salicina*	unknown	EU128065	EU128107
	CBS 100397	Italy	*Vitis vinifera*	S. Serra	AF246806	AY735498
	CBS 110703	South Africa	*V. vinifera*	L. Mostert	DQ173094	DQ173115
	STE U 6089	South Africa	*Prunus salicina*	Unknown	EU128063	EU128105
	P12	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605013	HQ605002
	P14	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605014	HQ605003
	P16	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605015	HQ605006
	P22	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605016	HQ605007
	P28	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605017	HQ605004
	P29	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605018	HQ605008
	P49	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605024	HQ605005
Pm. alvesii	CBS 110034	Brasil	Human	S.H. Alves	AY579301	AY579234
Pm. amstelodamense	CBS 110627	Netherlands	Human	J. Bruins	AY579295	AY579228
Pm. angustius	CBS 114992	U.S.A	*V. vinifera*	P. Larignon	DQ173104	DQ173127
Pm. australiense	CBS 113589	Australia	*V. vinifera*	T. Knaggs	AY579296	AY579229
	STE-U 5960	South Africa	*P. salicina*	Unknown	EU128069	EU128111
	STE-U 5961	South Africa	*P. salicina*	Unknown	EU128070	EU128112
Pm. cinereum	CBS 123909	Spain	*V. vinifera*	H. Mohammadi	FJ517157	FJ517149
Pm. croatiense	CBS 123037	Croatia	*V. vinifera*	B. Cvjetkovic	EU863482	EU863514
Pm. fuscum	STE-U 5969	South Africa	*P. salicina*	U. Damm	EU128098	EU128141
	STE-U 6366	South Africa	*P. salicina*	U. Damm	EU128199	EU128140
Pm. griseorubrum	STE-U 5957	South Africa	*P. salicina*	Unknown	EU128074	EU128116
	STE-U 5958	South Africa	*P. salicina*	Unknown	EU128075	EU128117
	CBS 111657	U.S.A	Human	D. Sutton	AY579294	AY579227

continues

Table1. *continued*

Species	Isolate number[a]	Origin	Host	Collector	GenBank accession numbers	
Pm. hispanicum	CBS 123910	Spain	*V. vinifera*	D. Gramaje	FJ517164	FJ517156
	P30	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal-Tebbal	HQ605019	HQ604996
Pm. hungaricum	CBS 123036	Hungary	*V. vinifera*	B.T. Dula	EU863483	EU863515
Pm. inflatipes	CBS 166.75	Costa Rica	*Nectandra sp.*	I.A.S. Gibson	AY579322	AY579258
	CBS 113273	U.S.A	*Hypoxylon truncatum*	B. Horn	AY579323	AY579260
Pm. iranianum	STE-U 6091	South Africa	*Prunus armeniaca*	Unknown	EU128078	EU128120
	CBS 101357	Italy	*Actinidia chinensis*	F. Calzarano & S. Di Marco	DQ173096	DQ173120
Pm. krajdenii	CBS 109479	Canada	Human	S. Krajden	AY579330	AY579267
Pm. pallidum	STE-U 6104	South Africa	*P. armeniaca*	U. Damm	EU128103	EU128144
Pm. parasiticum (Togninia parasitica)	CBS 860.73	U.S.A	Human	R.T. Steigbigel	AF246803	AY579253
	P37	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605020	HQ604998
	P39	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605021	HQ605000
	P46	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605022	HQ605001
	P56	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605010	HQ604999
	P62	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605023	HQ604997
	STE-U 6093	South Africa	*P. armeniaca*	Unknown	EU128081	EU128123
Pm. prunicolum	STE-U 5967	South Africa	*P. salicina*	U. Damm	EU128095	EU128124
	STE-U 5968	South Africa	*P. salicina*	U. Damm	EU128096	EU128138
Pm. rubrigenum (Tognina rubrigena)	CBS 498.94	U.S.A	Human	K.J. Kwon-Chung	AF246802	AY579238
Pm. scolyti	STE-U 6096	South Africa	*P. armeniaca*	Unknown	EU128084	EU128126
	STE-U 6099	South Africa	*Prunus persica*	Unknown	EU128087	EU128129
	STE-U 5954	South Africa	*P. salicina*	Unknown	EU128090	EU128132
Pm. sphinctrophorum	CBS 337.90	Laos U.S.A	Human	S. Krajden & R.C. Summerbell	DQ173113	DQ173142
Pm. subulatum	STE-U 6094	South Africa	*Prunus armeniaca*	Unknown	EU128092	EU128134

continues

Table1. *continued*

Species	Isolate number[a]	Origin	Host	Collector	GenBank accession numbers	
	CBS 113584	South Africa	*V. vinifera*	L. Mostert	AY579298	AY579231
Pm. tardicrescens	CBS 110573	U.S.A	Human	Levi	AY579300	AY579233
Pm. theobromatis	CBS 111586	Ecuador	*Theobroma gileri*	H.C. Evans	DQ173106	DQ173132
Pm. tuscanum	CBS 123033	Italy	*V. vinifera*	L. Mugnai	EU863458	EU863490
Pm. venezuelense	CBS 651.85	Venezuela	Human	M.B. De Albornoz	AY579320	AY579256
	CBS 110119	South Africa	*V. vinifera*	L. Mostert	AY579318	AY579251
	P1	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605011	HQ604993
	P4	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605012	HQ604995
	P6	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605026	HQ605009
	P8	Algeria. Tipaza	*V. vinifera*	A. Berraf-Tebbal	HQ605025	HQ604994
Pm. vibratilis	CBS 117115	Unknown	Unknown	Unknown	DQ649063	DQ649064
Pm. viticola	CBS 428.95	Germany	*Sorbus intermedia*	K. Weise	DQ173107	DQ173133
	CBS 113065	South Africa	*V. vinifera*	L. Mostert	DQ173105	DQ173128
T. africana	STE-U 6177	South Africa	*P. armeniaca*	U. Damm	EU128100	EU128142
	STE-U 6364	South Africa	*P. armeniaca*	U. Damm	EU128101	EU128143
T. austroafricana	CBS 112949	South Africa	*V. vinifera*	L. Mostert	DQ173099	DQ173122
T. fraxinopennsylvanica (Pm. mortoniae)	STE-U 6101	South Africa	*P. salicina*	Unknown	EU128079	EU128121
	STE-U 6102	South Africa	*P. salicina*	Unknown	EU128080	EU128122
T. griseo-olivacea	STE-U 5966	South Africa	*P. armeniaca*	U. Damm	EU128097	EU128139

[a] CBS, Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Diversity Centre, Utrecht, The Netherlands; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa.

Results

Isolation and identification of *Phaeoacremonium* species

A total of 61 isolates of *Phaeoacremonium* species were obtained from the 200 vines sampled. All isolates were typical *Phaeoacremonium* species with slow-growing colonies that gave visible growth after up to 15 days of incubation. The macroscopic features of the colonies such as colour, texture of the mycelium and the presence of pigment were used for preliminary identification. The isolates selected for molecular analysis and strains of *Phaeoacremonium* used for comparison are shown in Table 1.

A variability analysis was done to assess the genetic diversity within the *Phaeoacremonium* isolates. The bands produced by the MSP-PCR profiles divided the isolates into 9 meaningful groups with a reproducibility level of 80% (Figure 1). Representative isolates from each group including, when possible, isolates from Eutypa dieback and esca symptoms were selected for phylogenetic analysis.

The TUB and ACT sequences of the 17 isolates selected from the MSP-PCR profiles were combined and aligned with sequences of 50 isolates retrieved from GenBank, representing a selection of all known *Phaeoacremonium* species. The combined alignment consisted of 854 characters (including alignment gaps). Of these, 388 were parsimony informative, 74 were variable and parsimony uninformative and 392 were constant. After a heuristic search 4 parsimonious trees with the same overall topology were retained (length = 1614; CI = 0.511; RI = 0.857, HI = 0.489). One of the trees is shown in Figure 2. The isolates obtained in this study clustered with four previously published species, namely, *Pm. aleophilum*, *Pm. venezulense, Pm. parasiticum, Pm. hispanicum*.

Frequency and location of the *Phaeoacremonium* species

By relating the identities of representative isolates, based on β-tubulin and ACT sequence data, to the MSP-PCR groupings we determined the frequency of the different species in the sample of 61 isolates. *Phaeoacremonium aleophilum* was the most frequent species, followed by *Pm. parasiticum* and *Pm. venezuelense*. Only one isolate corresponded to *Pm. hispanicum*. *Phaeoacremonium* species occurred in 38 of the 100 vines showing Eutypa dieback symptoms and in 23 of the 100 vines showing esca (Table 2). Their incidence was much greater in the trunk than in the arms of the vines (Table 2). Among the four types of wood alteration (V-shaped necrosis, central necrosis, wood decay, and black spots), *Phaeoacremonium* species were most frequently isolated from V-shaped necroses (Table 2).

Discussion

Grapevine decline and the associated pathogens have been little studied in Algeria. This study constitutes the first attempt to assess the diversity of *Phaeoacremonium* species on grapevines showing Eutypa dieback and esca symptoms. Species identity was based on morphological characters and analysis of partial sequences of β-tubulin and actin genes. Four species were identified, namely *Pm. aleophilum*, *Pm. parasiticum*, *Pm. venezuelense* and *Pm. hispanicum*.

Phaeoacremonium aleophilum was the most frequently isolated species with an incidence of 68.8% of all the isolations. Interestingly it was mostly associated with V-shaped sectorial necrosis. This species is recognized as the most common species on grapevines worldwide (Mostert *et al.,* 2006b; Essakhi *et al.,* 2008; Gramaje *et al.,* 2009) and is most frequently associated with foliar symptoms of esca (Larignon and Dubos, 1997; Essakhi *et al.,* 2008, Péros *et al.,* 2008).

The next most frequent species were *Phaeoacremonium parasiticum* and *Phaeoacremonium venezuelense*. *Phaeoacremonium parasiticum* is well-known on grapevines and has been isolated in relatively high frequencies. It is also found on other woody hosts as an endophyte or as agent of plant disease (Mostert *et al.,* 2006b). *Phaeoacremonium parasiticum* is the most common species causing human infection, and was first reported in 1974 as *Phialophora parasitica* (Ajello *et al.,* 1974). It can be identified easily by its distinct dense mycelium and prominent exudate droplets, which are perceived as warts on the mycelium.

It was interesting to find such a high proportion of *Pm. venezuelense* on Algerian grapevines. This species has rarely been encountered on grapevines and is represented worldwide by only five strains, of which three were from human infections; the fourth was from a grapevine and the

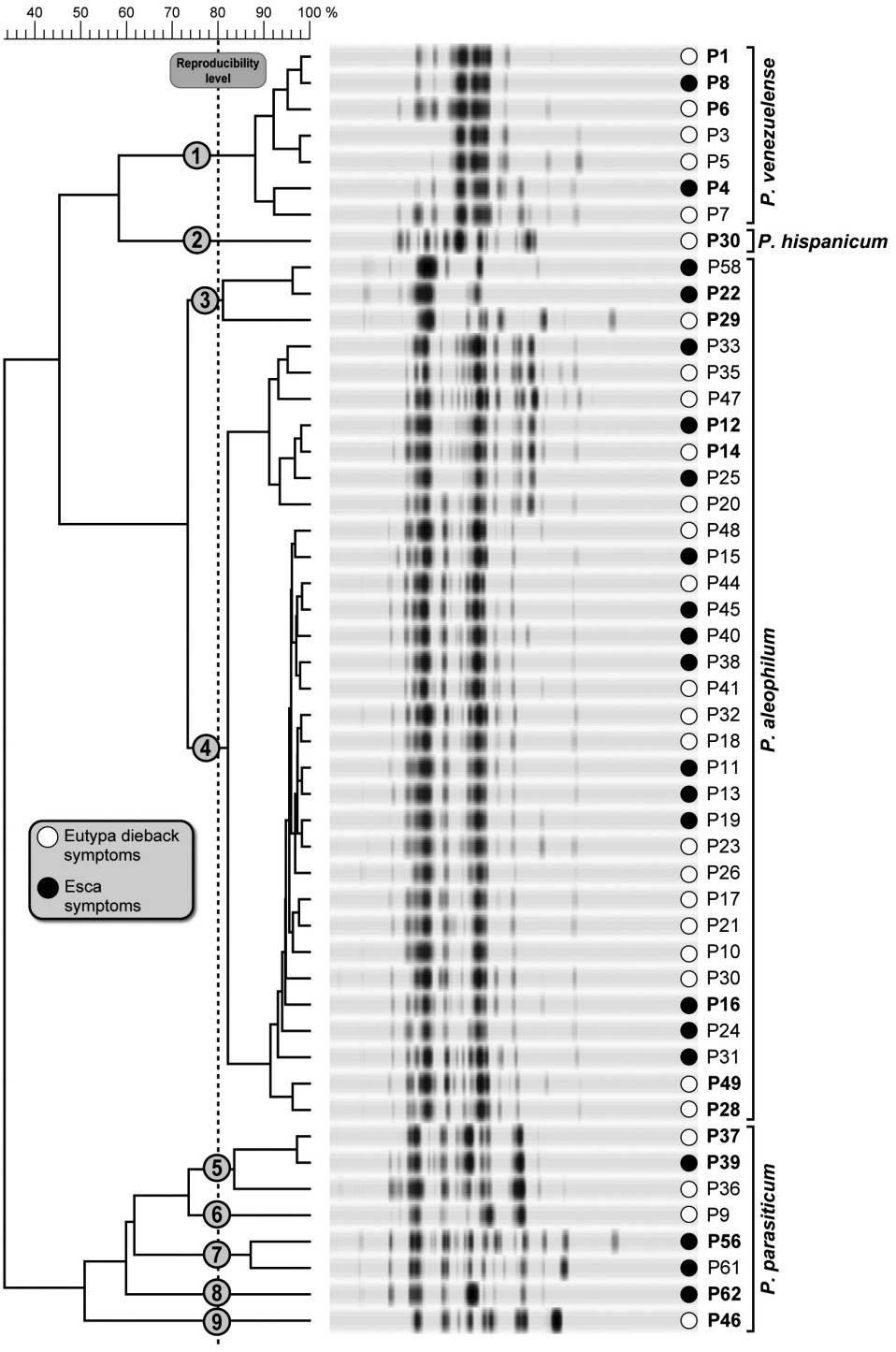

Figure 1. Consensus dendrogram from MSP-PCR profiles obtained with primer M13. The vertical dashed line corresponds to the reproducibility level (80%) from which nine groups of isolates are inferred (indicated by numbered circles). In each group, the isolates highlighted in boldface were selected for phylogenetic analysis. All fingerprints were grouped by similarity using the Pearson correlation coefficient and UPGMA. Isolates obtained in this study from vines with eutypa dieback or esca symptoms are indicated by white and black circles respectively.

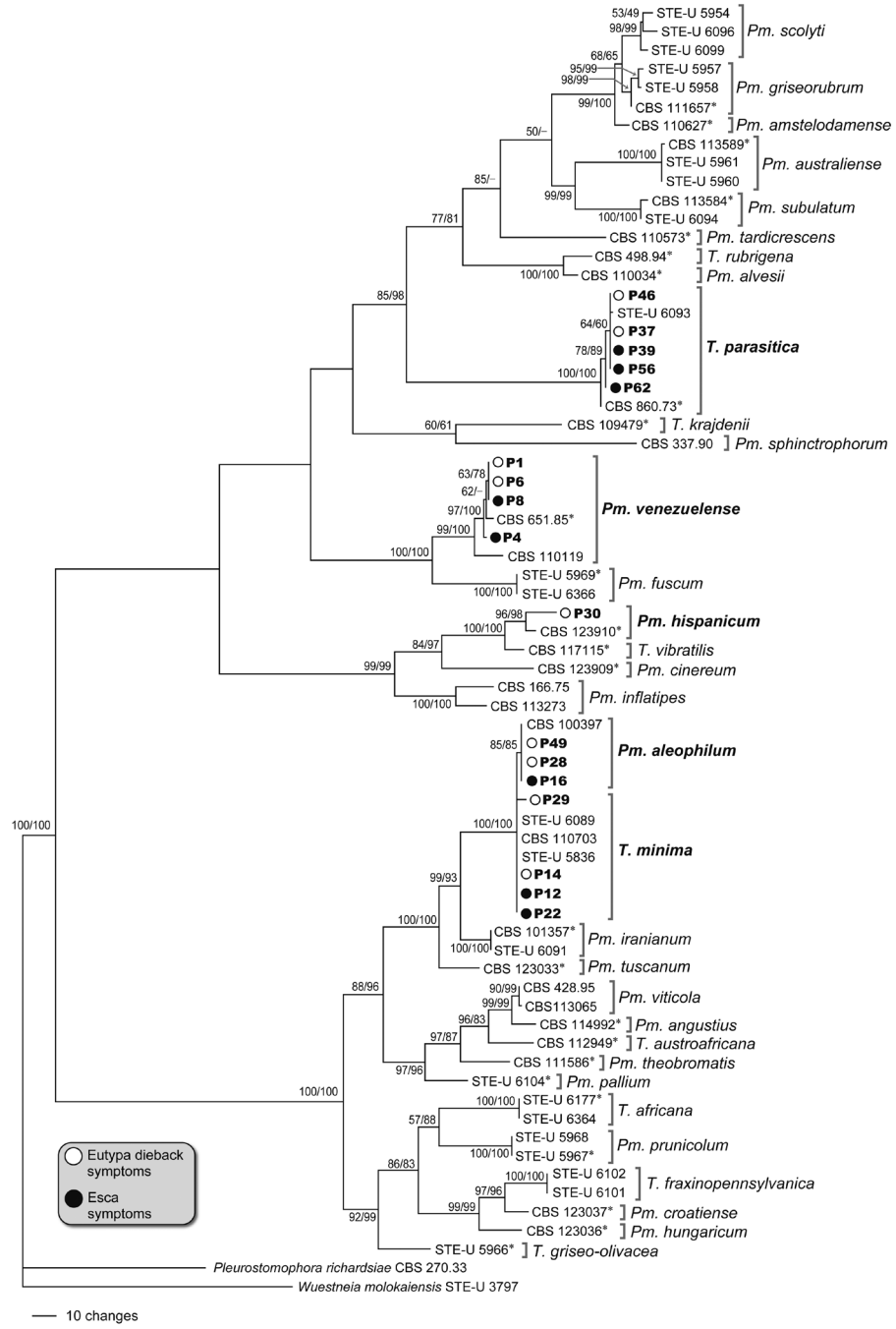

Figure 2. One of 4 equally parsimonious trees resulting from the alignment of 854 characters of combined *TUB* and *ACT* partial sequences. Length = 1614; consistency index (CI) = 0.511; retention index (RI) = 0.857; homoplasy index (HI) = 0.489. Newly generated sequences are highlighted in boldface and listed by their isolate number. Ex-type cultures are marked with an asterisk. Isolates obtained in this study from vines with eutypa dieback or esca are marked with white and black circles respectively. Bootstrap values from 1000 replications are shown for Maximum Parsimony (MP) and Neighbour-Joining (NJ) at the tree nodes (MP/NJ). Branches marked with a minus (–) are not present in the NJ tree. *Pleurostomophora richardsiae* (Genbank ACT: AY579271; TUB: AY579334) and *Wuestneia molokaiensis* (Genbank ACT: AY579335; TUB: AY579272) were included as outgroups.

Table 2. Fungal species isolated from wood lesions of grapevine trunks and arms.

Plant portion/species		Eutypa dieback				Esca			
		V-shaped necrosis	Central necrosis	Black spots	Wood decay	V-shaped necrosis	Central necrosis	Black spots	Wood decay
Trunks	*Phaeoacremonium aleophilum*	13	5	1	5	7	1	4	4
	Pm. parasiticum	2	1	0	1	2	0	2	1
	Pm. venezuelense	2	2	0	2	2	0	0	0
	Pm. hispanicum	0	0	0	0	0	0	0	0
Arms	*Pm aleophilum*	2	0	0	0	0	0	0	0
	Pm. parasiticum	0	0	0	1	0	0	0	0
	Pm. venezuelense	0	0	0	0	0	0	0	0
	Pm. hispanicum	1	0	0	0	0	0	0	0
Total		20	8	1	9	11	1	6	5

fifth strain from an unknown host (Guarro *et al.,* 2006). *Pm. venezuelense* was first described as *Cephalosporium serrae* in the first medical report involving *Phaeoacremonium* species (De Albornoz, 1974). Also of interest was the single isolate of *Pm. hispanicum*, which was described recently (Gramaje *et al.,* 2009) and has thus far been found only in Spain. *Phaeoacremonium hispanicum* can be identified by its distinct abundant percurrently rejuvenating conidiophores. It has the ability to grow at 37°C, which suggests that it has the potential to survive at human body temperature. This finding is quite interesting in relation to the ecology of *Pm. parasiticum* and *Pm. venezuelense*, as these thermotolerent species are associated with *Phaeohyphomycosis* in humans but have also been isolated from grapevines and other woody hosts (Mostert *et al.,* 2006a). According to these authors, *Phaeoacremonium* infections in humans appear to have become more common over the last two decades. Essakhi *et al.* (2008) isolated *Phaeoacremonium* species previously associated with human infections from the branches and trunks of *Vitis vinifera* with esca symptoms. However, the clinical importance of *Pm. hispanicum* remains to be determined.

The majority of *Phaeoacremonium* species have been isolated from diseased woody plants. With three new species recently described by Graham *et al.* (2009), the number of *Phaeoacremonium* species reported on grapevine worldwide has now reached 25. The two main diseases in which these species are involved are esca and Petri disease the latter formerly known as *Phaeoacremonium* grapevine decline affecting young vines (Mugnai *et al.,* 1999; Mostert *et al.,* 2006b; Luque *et al.,* 2009). Inoculation studies have shown that *Pm. aleophilum* causes brown streaking, reduced shoot growth and esca symptoms on grapevine leaves and berries (Gubler *et al.,* 2004b). Similar studies have shown that *Pm. parasiticum*, *Pm. krajdenii*, *Pm. subulatum*, *Pm. venezulense* and *Pm. viticola* also cause brown wood streaking (Halleen *et al.,* 2005). However, our study clearly demonstrated that in Algeria *Phaeoacremonium* species were mainly isolated from vines showing the typical external and internal symptoms of eutypa dieback. How far these species are also involved in Eutypa dieback is not known, and this topic should be studied. *Phaeoacremonium* species were mostly isolated from V-shaped (sectorial) necrosis; which is not consistent with the literature, which reports that they occur in central brown lesions (Larignon and Dubos, 1997; Péros *et al.,* 2008, Luque *et al.,* 2009). In our study these species were much more common in the trunks than in the arms, suggesting that the infections they caused were derived from mother material or from the nursery. On the contrary, Luque *et al.* (2009) isolated *Phaeoacremonium* species more frequently from the arms than from the trunks, which would indicate that in-

fection occurred through wounds caused by annual pruning. This suggestion was made by Rego *et al*. (2000) and also by Gubler *et al*. (2004a) and Larignon (2004), but further studies are needed to confirm them.

This work highlights the importance of the genus *Phaeoacremonium* on grapevines in Algeria. It also indicates that in general, the effects that fungi have on the health of Algerian grapevines should be studied in greater detail.

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Literature cited

Ajello L., L.K. Georg, R.T. Steigbigel and C.J.K. Wang, 1974. A case of phaeohyphomycosis caused by a new species of *Phialophora*. *Mycologia* 66, 490–498.

Altschul S.F., W. Gish, E.W. Miller and D.J. Lipman, 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215, 403–410.

Alves A., I. Henriques, S. Fragoeiro, C. Santos, A. J. L. Phillips and A. Correia, 2004. Applicability of rep-PCR genomic fingerprinting to molecular discrimination of members of the genera *Phaeoacremonium* and *Phaeomoniella*. *Plant Pathology* 53, 629–634.

Anonymous, 2009. *D.S.A.S.I and M.A.D.R. Direction des Statistiques Agricoles et des Systèmes d'Information*. Ministère de l'agriculture. Série B. Algérie.

Berraf A. and J.P. Péros, 2005. Importance de l'eutypiose et de l'esca en Algérie et structure de la communauté fongique associée. *Journal International des Sciences de la Vigne et du Vin* 39 (3), 121–128.

Carbone I. and L.M. Kohn, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91, 553–556.

Carter M.V., 1991. *The Status* of Eutypa lata *as a Pathogen*. Wallingford, UK, Commonwealth Agricultural Bureau, International Mycological Institute. *Phytopathological Paper* No. 32.

Crous P.W. and W. Gams, 2000. *Phaeomoniella chlamydospora* gen. et comb. nov., a causal organism of Petri grapevine decline and esca. *Phytopathologia Mediterranea* 39, 112–118.

Debraye F., 1892. Apoplexie de la vigne. *Progres Agricole et Viticole* 17, 528–531.

De Albornoz M.B., 1974. *Cephalosporium serrae*, agente etiologico de micetomas. *Mycopathologia et Mycologia Applicata* 54, 485–498.

Edwards J. and I.G. Pascoe, 2004. Occurrence of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* associated with Petri disease and esca in Australian grapevines. *Australasian Plant Pathology* 33, 273–279.

Essakhi S., L. Mugnai, P.W. Crous, J.Z. Groenewald and G. Surico, 2008. Molecular and phenotypic characterization of novel *Phaeoacremonium* species associated with Petri disease and esca of grapevine. *Persoonia* 21, 119–134.

Ferreira J.H.S., F.N. Matthee and A.C. Thomas, 1989. Fungi associated with dieback and pruning wounds of grapevines in South Africa. *South African Journal of Enology and Viticulture* 10, 62–66.

Fischer M., 2002. A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycological Progress* 1, 315–324.

Gams W., G.J.M. Verkley and P.W. Crous (eds.), 2007. *CBS Course of Mycology*. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Glass N.L. and G.C. Donaldson, 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61, 1323–1330.

Graham A.B., P. R. Johnston and B. S. Weir, 2009. Three new *Phaeoacremonium* species on grapevines in New Zealand. *Australasian Plant Pathology* 38, 505–513.

Gramaje D., J. Armengol, H. Mohammadi and L. Mostert, 2009. Novel *Phaeoacremonium* species associated with Petri disease and esca of grapevine in Iran and Spain. *Mycologia* 101, 920–929.

Guarro J., A.M. Silvestre, G. Verkley, J. Cano, O.F. Gompertz, G. Gene, M.L.M. Ogawa, J.T. Tamashita, S.P. Teixeira and F.A. Almeida, 2006. Limitations of DNA sequencing for diagnosis of a mixed infection by two fungi, *Phaeoacremonium venezuelense* and a *Plectophomella* sp., in a transplant recipient. *Journal of Clinical Microbiology* 44, 4279–4282.

Gubler W.D., K. Baumgartner, G.T. Browne, A. Escalen, L.S. Rooney, E. Petit and L.A. Bayramian, 2004a. Root diseases of grapevine in California and their control. *Plant Pathology* 33, 157–165.

Gubler W.D., S. Torlachen Thind, J. A. Feliciano and A. Eskalen, 2004b. Pathogenicity of *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* on grape berries in California. *Phytopathologia Mediterranea* 43, 70–74.

Halleen F., L. Mostert and P.W. Crous, 2005. Pathogenicity testing of *Phialophora, Phialophora*-like, *Phaeoacremonium* and *Acremonium* species isolated from vascular tissues of grapevines. In: *Proceedings of the 4th International Workshop on Grapevine Trunk Diseases*, Stellenbosch, South Africa, 58 pp.

Hall T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows

95/98/NT. *Nucleic Acids Symposium Series* 41, 95–98.
Hildebert I., 1949. Vigne et colonisation en Algérie. *Annales de Géographie* 58, 212–219.
Larignon P. and B. Dubos, 1997. Fungi associated with esca disease in grapevine. *European Journal of Plant Pathology* 103, 147–157.
Larignon P., 2004. Réflexions sur l'esca. Ce qu'on l'on sait déjà montre qu'il en reste beaucoup à apprendre. *Phytoma* 576, 28–31.
Luque J., M. Martos, A. Aroca, R. Raposo and F. Garcia-Figueres, 2009. Symptoms and fungi associated with decling mature grapevine plants in northeast Spain. *Journal of Plant Pathology* 91, 381–390.
Meyer W., T.G. Mitchell, E.Z. Freedman and R. Vilgalis, 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. *Journal of Clinical Microbiology* 31, 2274–2280.
Moller W.S. and A.N. Kasimatis, 1978. Dieback of grapevines caused by *Eutypa armeniacae*. *Plant Disease Reporter* 62, 254–258.
Mostert L., F. Hallen, P. Fourie and P.W. Crous, 2006a. A review of *Phaeoacremonium* species involved in Petri diseases and esca of grapevine. *Phytopathologia Mediterranea* 45 (Supplement) S12–S29.
Mostert L., J.Z. Groenewald, R.C. Summerbell, W. Gams and P.W. Crous, 2006b. Taxonomy and Pathology of *Togninia* (*Diaporthales*) and its *Phaeoacremonium* anamorph. *Studies in Mycology* 54, 1–115.
Mugnai L., A. Graniti and G. Surico, 1999. Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevine. *Plant Disease* 83, 404–416.
O'Donnell K. and E. Cigelnik, 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7, 103–116
Page R.D.M., 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12, 357–358.
Pascoe, I.G. and E. Cottral, 2000. Development of grapevine trunk disease research in Australia. *Phytopathologia Mediterranea* 39, 68–75.
Péros J.P., G. Berger and I. Jamaux-Despreaux, 2008. Symptoms, wood lesions and fungi associated with esca in organic vineyards in Languedoc-Rousillon (France). *Journal of Phytopathology* 156, 297–303.
Phillips A.J.L., 2002. *Botryosphaeria* species associated with diseases of grapevines in Portugal. *Phytopathologia Mediterranea* 41, 3–18.
Ravaz L., 1905. Sur la cause du dépérissement des vignes de la Tunisie, de l'Algérie et du Midi de la France. *Comptes Rendus de l'Académie des Sciences*, *Paris* 141, 58–59.
Rayner R.W. (ed.), 1970. *A Mycological Colour Chart*. Commonwealth Mycological Institute and British Mycological Society. Kew, Surrey, UK.
Rego C., H. Oliviera, A. Carvalho and A.J.L. Phillips, 2000. Involvement of *Phaeoacremonium spp*. and *Cylindrocarpon destructans* with grapevine decline in Portugal. *Phytopathologia Mediterranea* 39, 76–79.
Santos J.M. and A.J.L. Phillips, 2009. Resolving the complex of Diaporthe (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. *Fungal Diversity* 34, 111–125.
Swofford DL. (ed.) 2003. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods) v4*. Sinauer Associates, Sunderland, MA, USA.
Thompson J.D, T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876–4882.
Viala P., 1926. Recherche sur les maladies de la vigne: esca. *Annales des Epiphyties* 12, 1–108.

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