

RESEARCH PAPERS

***Phaeoacremonium* and *Botryosphaeriaceae* species associated with cypress (*Cupressus sempervirens* L.) decline in Kerman province (Iran)**

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Summary. Common cypress (*Cupressus sempervirens* L.) is an east Mediterranean plant element and one of four native conifers in Iran. During spring and summer of 2012, a field survey was carried out in different areas of Kerman province (south-eastern Iran) to study cypress decline diseases. Samples were collected from crowns, trunks and branches of cypress trees showing yellowing, dieback, canker, wilting of leaves and internal wood discoloration. Isolations were made from symptomatic wood tissues. Based on morphological and molecular characteristics, four species of *Phaeoacremonium*, namely *Phaeoacremonium parasiticum*, *Pm. aleophilum*, *Pm. iranianum* and *Pm. rubrigenum*, and two species of the *Botryosphaeriaceae*, *Botryosphaeria dothidea* and *Neofusicoccum parvum*, were isolated and identified. Pathogenicity tests were undertaken to determine the role of these species on 2-year-old potted cypress plants and green shoots of grapevine. *Neofusicoccum parvum* was more virulent than the other species and caused the largest lesions on both hosts. The fungi were re-isolated from margins of lesions and healthy tissue, thus completing Koch's postulates. This is the first report of *B. dothidea*, *N. parvum*, *Pm. aleophilum*, *Pm. rubrigenum* and *Pm. iranianum* as pathogens on Mediterranean cypress trees.

Key words: β -tubulin, internal transcribed spacers, trunk disease.

Introduction

The genus *Cupressus* L. (Cupressaceae) includes as many as 25 species, largely distributed in the Mediterranean basin, in Asia and North America (Giovannelli and De Carlo, 2007). The natural distribution of *Cupressus sempervirens* L. is characterized by disjunction, and often relic populations are growing in Iran, Syria, Jordan, Libya, Aegean Islands, Crete, Turkey and Cyprus (Zohary, 1973). In the Mediterranean region, *C. sempervirens* is an important forest species used for multiple purposes because of its ability to grow in adverse environments such as calcareous, clay, dry and poor soils

(Gallis *et al.*, 2006); it has an important role in the landscape, local economy, symbolism and culture (Bagnoli *et al.*, 2009). Several fungi of genera such as *Seiridium* Nees: Fr. are well-known pathogens of Cupressaceae; *S. cardinale* is seriously threatening the survival of these trees in Mediterranean countries (Panconesi, 1990; Graniti, 1998). *Seiridium* canker is the best known disease of Cupressaceae and has a wide geographical distribution (Boesewinkel, 1983; Solel *et al.*, 1983; Vander Werff, 1988), but various other fungal diseases of cypress trees have been recorded in some countries. These include *Pestalotiopsis* canker caused by *Pestalotiopsis funerea* (Desm.) Steyaert (Madar *et al.*, 1991), crown wilt, stem canker and seedling blight caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (Bruck *et al.*, 1990) and *Diplodia* canker of *C. sempervirens* caused by *Diplodia cupressi* A.J.L. Phillips & A. Alves [syn. *Diplodia pinea*

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(Desm.) J. Kickx f. sp. *Cupressi*] (Solel *et al.*, 1987; Alves *et al.*, 2006). In South Africa *Sphaeropsis* sp. and *Seiridium unicorne* (Cooke & Ellis) B. Sutton were reported as causal agents of cypress canker (Linde *et al.*, 1997). During an investigation by Abdollahzadeh *et al.* (2009), a new species of the Botryosphaeriaceae, *Phaeobotryon cupressi* Abdollahzadeh, Zare & A.J.L. Phillips, was isolated from *C. sempervirens* trees in Gorgan (Golestan province, north-eastern Iran), but pathogenicity of this fungus was not tested. Despite the importance attributed to the species of *Phaeoacremonium* and Botryosphaeriaceae on grapevine in Iran, there have been no studies on the role of these species on other woody trees such as cypress in this country. Although *C. sempervirens* is native to Iran, little attention has been given to *Cupressus* spp. as hosts of fungal trunk pathogens. In spring of 2012 yellowing and dieback of cypress trees was noticed in Kerman. On closer examination, different internal symptoms were observed in cross sections of wood from affected trees. The present study was therefore undertaken to isolate and identify *Phaeoacremonium* and Botryosphaeriaceae species associated with cypress decline in this region of Iran.

Materials and methods

Sampling and fungal isolation

In May 2012, severe yellowing and dieback of cypress trees was noticed in Kerman (south-eastern Iran). Symptomatic trees were located in an urban park established approximately 40 years previously. Additional samples were collected from cypress trees planted as ornamentals in parks, along streets and as shade trees in Mahan, Jiroft, Sirch, Ravar, Pariz and Sirjan, in this province. Samples were collected from trunks, crowns and branches of cypress trees showing different disease symptoms, including canker, yellowing, wilting of leaves on several branches, and different symptoms in wood, including brown to black spots, brown internal necrosis, brown to black streaking, wedge-shape necrosis and watery necrosis. The superficial bark from each piece was removed and ten thin cross sections (6–7 mm thick) were cut from symptom-bearing tissue. About ten wood tissue pieces (about 5×5×5 mm) were taken from the margins between necrotic and apparently healthy tissues. Wood pieces were immersed in 1.5% sodium hypochlorite solution for 60 sec, washed three times

with sterile distilled water and plated onto malt extract agar (MEA; 2%, Merck) supplemented with 100 mg L⁻¹ streptomycin sulphate (MEAS). Plates were incubated at 25°C in the dark until growth could be detected. Subcultures were made from the growing hyphae onto potato dextrose agar (PDA; Merck) or MEA plates. Single conidium cultures were obtained prior to morphological and molecular identification of the fungi.

Fungal identification

Morphological identification

Phaeoacremonium species were identified based on culture characters and pigment production on PDA, MEA and oatmeal agar (OA; 30 g oatmeal; 15 g agar; Merck). Microscopic observations of phialide type and shape, conidiophore morphology and hyphal wart size from aerial mycelium were made on MEA. Radial growth of isolates was measured after 16 d at 25°C (Mostert *et al.*, 2006). Species of the Botryosphaeriaceae were identified according to colony and conidial morphology (Van Niekerk *et al.*, 2004). In order to enhance sporulation, pure cultures were placed on 2% water agar (WA, 2% agar; Merck) containing autoclaved pine needles, and incubated at 25°C under near-UV (light/darkness for 12/12 h). Isolates were examined weekly for formation of pycnidia and conidia. Fifty microscopic measurements of each type of fungal structure were made for all isolates.

Molecular identification

Isolates were grown on PDA for 2 weeks at 25°C in the dark. For each isolate, approximately 50 mg of fungal mycelium was scraped from the culture surface and mechanically disrupted by grinding to a fine powder under liquid nitrogen using a mortar and pestle. Total DNA was extracted using the Peq Gold Fungal DNA mini Kit (Roche) following the instructions of the manufacturer. DNA samples were kept at -20°C until used for PCR amplification. The β -tubulin gene was amplified for the strains identified as *Phaeoacremonium* as described by Mostert *et al.* (2006) using primer sets T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995).

For the Botryosphaeriaceae isolates, the internal transcribed spacers (ITS1 and ITS2) and the 5.8S ribosomal gene were amplified using the primer pair ITS4 and ITS5 (White *et al.*, 1990) as described by

Úrbez-Torres *et al.* (2008). PCR amplifications were performed on an iCycler thermal cycler (Bio-Rad). The PCR products were visualized on 1% agarose gels (UltraPure™ Agarose, Invitrogen). A 100 bp ladder was used as a molecular weight marker (GeneRuler™ DNA Ladder Mix, Fermentas). PCR products were purified with the High Pure PCR Product Purification Kit (Bioneer) and sequenced in both directions by Macrogen Inc. Sequencing Center (Seoul, South Korea).

Pathogenicity tests

Six isolates, *Pm. aleophilum* (PACYP1, GenBank accession No. KC480187), *Pm. parasiticum* (PMP-CYP1, GenBank KC467058), *Pm. iranianum* (PMICYP1, GenBank KC416211), *Pm. rubrigenum* (PRCYP1, GenBank KC416210), *N. parvum* (NPCYP1, GenBank KC467060) and *B. dothidea* (BDCYP1, GenBank KC467062), were used for pathogenicity tests. Artificial inoculations were conducted on 2-year-old potted plants (about 150 cm in height). For each isolate, four plants, showing neither foliar symptoms nor wood deterioration, were chosen and the outer bark at the inoculation areas was cleaned and sprayed with 70% ethanol. A superficial wound (5×5 mm, reaching into the xylem) was made on the stem of each plant with a sterilized scalpel. A mycelial plug (5 mm diam.) obtained from the margin of a fungal colony was placed in the wound with the mycelium facing towards the stem, and the wound was wrapped with Parafilm® (Pechiney Plastic Packaging). Control plants were inoculated with sterile PDA plugs instead of the fungal inoculum. Inoculated plants were placed in a completely randomized design in a glasshouse at approximately 25°C. Three months after inoculation, the length of the internal vascular lesions was recorded, by removing the bark from the stem of each plant and measuring the necrotic lesions above and below the inoculation site. Surface-sterilized wood pieces taken from necrotic tissues were plated on PDA to re-isolate inoculated fungi so as to fulfill Koch's postulates. One-way analysis of variance (ANOVA) in SAS v 9.1 (SAS Institute) was performed to evaluate differences in the extent of vascular discolorations. The LSD test was used for comparison of treatment means at $P < 0.01$. A second pathogenicity test, using the same isolates was conducted on green shoots of grapevine (cv. Askari). Fifty-six green shoots of each of approximately 30

cm length were cut from vines from Experimental vineyards of the University of Shahid Bahonar, Kerman, Iran, and immediately inoculated as described above, eight per fungal isolate. Inoculated shoots were placed in sterile water and maintained for 20 d at room temperature. Afterwards, the shoots were sectioned longitudinally, vascular discoloration was recorded, and green tissue re-isolations were made as described above.

Results

Survey and sample collection

Forty nine samples collected from different parts of Kerman province were studied (Table 1). Fungal trunk pathogens were isolated from 41 samples (84%). Affected cypress trees showed different symptoms including yellowing, canker, wilting of leaves on several branches, dieback and black spots, brown internal necrosis, watery necrosis and brown to black streaking in cross sections (Figure 1). Some plants, especially in Kerman and Sirjan, showed severe decline symptoms and eventually died. In Sirch and Mahan, approximately 20% of infected trees displayed elongated cankers while in an urban park in Kerman (Ghaem Park) about 30% of the cypress trees showed yellowing and wilting symptoms. One of the most common internal symptoms on diseased cypress trees were longitudinal brown to black streaks that appeared as necrotic black spots in cross sections. In some cases disease symptoms such as dieback and yellowing were observed only on one side of the trees or on lateral branches. On closer examination, wood discoloration was observed on affected branches in cross sections. In some areas such as Kerman and Sirjan, the trunk bases of withered trees showed circular necrosis. When the outer layer of these areas was scraped away, dark brown wood discoloration extended upwards for several centimeters around the affected tissues. In cross-sections the dark streaks on the wood tissue were often a few mm wide forming sectorial black cankers. In branches with canker symptoms, wedge-shaped wood discolorations and sectorial brown lesions were often observed.

Fungal isolation and identification

In this study 171 fungal isolates were recovered from cypress trees showing different external and in-

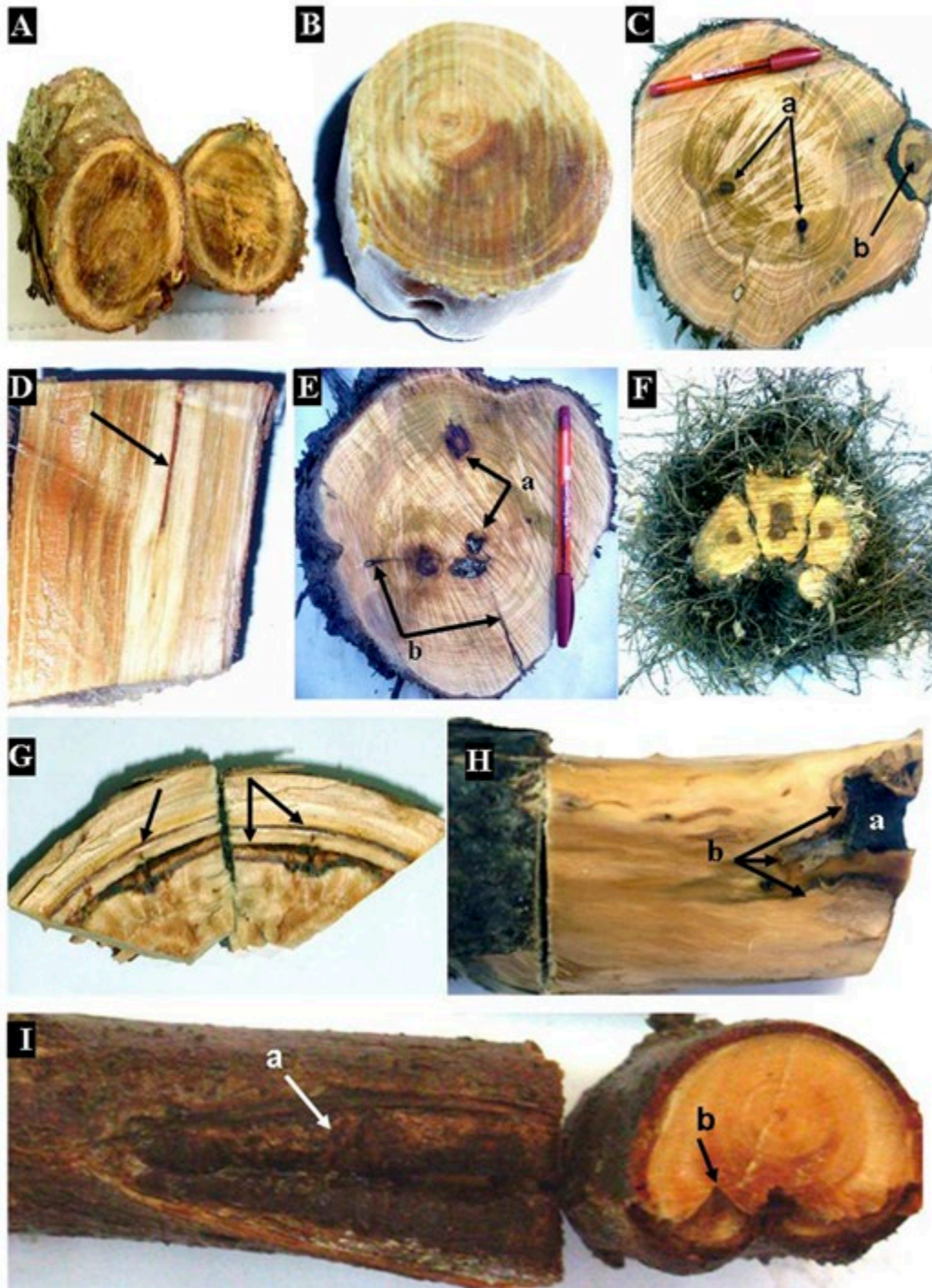


Figure 1. Symptoms associated with trunk disease on cypress trees. A, central wood necrosis. B, watery necrosis, C, co-occurrence of black spots (a) and central necrosis on a lateral branch (b). D, vascular discoloration as a brown line in longitudinal section, indicated by arrow. E, co-occurrence of black internal necrosis (a) and black wood streaking (b). F, internal wood necrosis at the crown of affected cypress trees. G, brown to black wood streaking in cross section. H, canker (a) and dark brown wood discolorations around the affected tissues of a canker (b). I, a canker in longitudinal (a) and cross section with wedge-shaped wood discoloration (b).

Table 1. Geographical origin, associated external and internal symptoms and number of fungal isolates recovered from cypress trees in Kerman province.

Fungal isolates		Isolation zone			External symptoms ^a	Internal lesion types ^b						Location
Identity	No. of isolates (%)	Crown	Stem	Branches		1	2	3	4	5	6	
<i>Phaeoacremonium parasiticum</i>	52 (30.4%)	+	+	+	Y,DI,D,W	22	12	8	7	1	2	Kerman, Mahan, Jiroft, Sirch, Ravar, Pariz, Sirjan
<i>Phaeoacremonium aleophilum</i>	16 (9.4%)	+	+	+	Y,DI,D	11	3	2	-	-	-	Sirjan, Mahan, Sirch, Kerman
<i>Phaeoacremonium iranianum</i>	3 (1.7%)	-	+	-	DI	2	-	1	-	-	-	Kerman
<i>Phaeoacremonium rubrigenum</i>	1 (0.6%)	-	-	+	Y	1	-	-	-	-	-	Kerman
<i>Neofusicoccum parvum</i>	8 (4.7%)	-	-	+	C,D	-	2	1	-	2	3	Sirch, Mahan
<i>Botryosphaeria dothidea</i>	7 (4.1%)	-	-	+	C, DI	-	1	1	-	1	4	Kerman, Sirjan
<i>Paecilomyces variotii</i>	18 (10.5%)	-	+	+	Y,DI,D	-	5	8	-	5	-	Kerman, Mahan, Jiroft,
<i>Alternaria</i> sp.	9 (5.3%)	+	+	+	Y,D	6	-	-	-	3	-	Kerman, Mahan, Jiroft
<i>Nattractia mangiferae</i>	11 (6.4%)	-	-	+	DI,D	3	8	-	-	-	-	Sirjan, Kerman
<i>Fusarium equiseti</i>	6 (3.5%)	-	+	-	D,DI	-	2	4	-	-	-	Kerman
<i>Fusarium</i> spp.	15 (8.8%)	+	+	+	Y,DI,D	-	9	6	-	-	-	Kerman, Jiroft, Sirch, Ravar, Sirjan
<i>Aspergillus</i> sp.	8 (4.7%)	-	-	-	DI,D	4	3	1	-	-	-	Sirjan, Mahan, Sirch
<i>Penicillium</i> sp.	11 (6.4%)	+	-	+	DI,D	5	4	-	-	2	-	Kerman, Mahan, Jiroft, Sirch, Ravar
<i>Phoma</i> sp.	6 (3.5%)	-	-	+	DI,D	2	3	-	-	-	-	Kerman, Jiroft

^a Summary of observed external symptoms: Y = yellowing, DI = dieback, D = decline, C = canker, W = wilting.

^b Summary of observed internal symptoms in the original materials: 1, brown to black spots; 2, brown internal necrosis; 3, brown to black streaking; 4, watery necrosis; 5, dark brown wood discoloration around the canker tissues; 6, wedge-shaped necrosis.

ternal disease symptoms. Four *Phaeoacremonium* species (Table 1) were found, namely *Pm. parasiticum* (52 isolates), *Pm. aleophilum* (16), *Pm. iranianum* (3) and *Pm. rubrigenum* (one), comprising 42.1% of the total fungal isolates recovered. β -Tubulin gene sequences of the four *Phaeoacremonium* isolates from Iran respectively showed 100% homology with *Pm. aleophilum* (isolate Pal-184, GenBank JQ044516, Gramaje *et al.*, 2013), 100% homology with *Pm. parasiticum*

(isolate P46, GenBank HQ605022, Berraf-Tebbal *et al.*, 2011), 99% homology with *Pm. iranianum* (strain Pir-4, GenBank FJ872406, Gramaje *et al.*, 2009b) and 100% homology with *Pm. rubrigenum* (strain 119Pal, GenBank EU863484, Essakhi *et al.*, 2008) deposited in GenBank. The ITS sequences of Botryosphaeriaceae isolates had 100% similarity with isolates previously identified as *N. parvum* (isolate CBS 121486, GenBank EU650672, Martos *et al.*, 2011) and *B. dothidea*

Table 2. Mean lesion length and re-isolation frequencies of fungal species inoculated into cypress stems (after 3 months) and green shoots of grapevine (after 20 days) in pathogenicity trials.

Fungal species	Strains inoculated			Mean lesion length (mm) ^b		Re-isolation frequency %	
	KER-U No. ^a	Code	Accession No.	Cypress	Grapevine	Cypress	Grapevine
<i>Phaeoacremonium aleophilum</i>	KRC-12	PACYP1	KC480187	20.45 b	28.88 b	66.6	91.6
<i>Phaeoacremonium parasiticum</i>	KRM-8	PMPCYP1	KC467058	18.50 b	23.25 c	75.0	83.3
<i>Phaeoacremonium iranianum</i>	KRS-1	PMICYP1	KC416211	13.20 c	24.87 c	41.7	33.3
<i>Phaeoacremonium rubrigenum</i>	KRK-1	PRCYP1	KC416210	7.50 d	10.80 e	50.0	16.7
<i>Neofusicoccum parvum</i>	KRM-5	NPCYP1	KC467060	23.45 a	34.87 a	83.3	91.6
<i>Botryosphaeria dothidea</i>	KRS-3	BDCYP1	KC467062	14.25 c	15.38 d	33.3	58.3
PDA plug				5.00 d	6.37 f		
LSD ($P < 0.01$)				2.75	3.41		

^a Culture collection of Plant Protection Department, College of Agriculture, University of Shahid Bahonar, Kerman, Iran.

^b Means with the same letter are not significantly different.

(isolate BOTDOT 1/4-150305-2, GenBank AJ938005, Jurc *et al.*, 2006). *Phaeoacremonium parasiticum* was the most frequently isolated species (30% of total isolates), while the other important isolates presented the following percentages: *Pm. aleophilum* (9%), *Pm. iranianum* (2%), *Pm. rubrigenum* (1%) *N. parvum* (5%) and *B. dothidea* (4%). *Phaeoacremonium parasiticum* was isolated from stems, crowns and branches of diseased trees showing different internal symptoms including brown to black spots (22 isolates), brown internal necrosis (12 isolates), brown to black streaking (eight isolates), wedge-shaped necrosis (two isolates), dark brown wood discoloration around canker tissues (one isolate), and watery necrosis (four isolates) of infected parts. *Phaeoacremonium aleophilum* was isolated from brown to black spots (11 isolates), brown to black streaking (two isolates) and brown internal necrosis (three isolates). Three isolates of *Pm. iranianum* were isolated from the stem of a 35-year-old cypress tree showing dieback symptoms in Kerman, viz. black spots (two isolates) and brown to black wood streaking (one isolate). Only one isolate of *Pm. rubrigenum* was obtained from a 30-year-old cypress tree in Kerman showing yellowing symptoms and black spots in branch cross sections. Of the Botryosphaeriaceae, eight *N. parvum* isolates were obtained from affected branches of cypress trees showing canker and decline symptoms in

Sirch and Mahan, viz. brown internal necrosis (two isolates), brown to black streaking (one isolate), dark brown wood discoloration around canker tissues (two isolates), and wedge-shaped necrosis (three isolates). Seven isolates of *B. dothidea* were also isolated from affected branches of cypress trees showing canker and dieback symptoms in Kerman and Sirjan, viz. brown internal necrosis (one isolate), brown to black streaking (one isolate), dark brown wood discoloration around the canker tissues (one isolate), and wedge-shaped branch necrosis (four isolates). A combination of *Pm. aleophilum* and *Pm. parasiticum* was isolated from only one symptomatic sample, from internal brown to black spots and brown internal necrosis in Sirjan. Other fungi, such as *Paecilomyces variotii*, *Nattrassia mangiferae*, *Fusarium equiseti*, other *Fusarium* spp., *Phoma* sp., *Alternaria* sp., *Aspergillus* sp., and *Penicillium* sp., were also occasionally isolated from different internal wood symptoms (Table 1).

Pathogenicity tests

The results of the pathogenicity tests (Table 2) showed that all isolates (with the exception of *Pm. rubrigenum*) were pathogenic on cypress trees ($F = 97.02$, $P < 0.0001$) and green shoots of grapevine ($F = 128.25$, $P < 0.0001$, ANOVA tables not shown). Three

months after inoculation, small brown rounded to elongated lesions (6 to 25 mm long) were visible on all inoculated cypress stems, whereas the control inoculations produced no lesions. *Neofusicoccum parvum* was the most virulent pathogen, with a mean lesion length of 23.5 mm. *Phaeoacremonium aleophilum* (20.5 mm), *Pm. parasiticum* (18.5 mm), *B. dothidea* (14.3 mm) and *Pm. iranianum* (13.2 mm) produced lesion lengths significantly longer than the negative controls (5.0 mm). Only with *Pm. rubrigenum* lesions were not significantly longer (7.5 mm) than in the negative controls. Lengths of lesions caused by *Pm. aleophilum* and *Pm. parasiticum* isolates were similar to each other, as were those produced by *Pm. iranianum* and *B. dothidea*. Re-isolation was successful, between 33% (*B. dothidea*) to 83% (*N. parvum*) (Table 2). Analyses of variance of the lesion lengths on inoculated grapevine shoots indicated significant treatment effects.

All the fungal isolates tested were pathogenic and produced extending internal vascular lesions on inoculated shoots. *Neofusicoccum parvum* was the most virulent and produced significantly ($P < 0.0001$) longer lesions (mean = 34.9 mm) in inoculated shoots than *Pm. aleophilum* (28.9 mm), *Pm. parasiticum* (23.2 mm), *Pm. iranianum* (24.9 mm), *B. dothidea* (15.4 mm) and *Pm. rubrigenum* (10.9 mm). No statistically significant difference in mean lesion length was found between *Pm. parasiticum* and *Pm. iranianum* isolates. *Phaeoacremonium rubrigenum* produced smaller lesions than the other isolates, but still produced lesions that were significantly longer than those of the controls (6.4 mm). All isolates were re-isolated from the inoculated shoots with frequencies between 33% (*Pm. iranianum*) to 92% (*N. parvum* and *Pm. aleophilum*). No Botryosphaeriaceae or *Phaeoacremonium* fungi were re-isolated from the control treatments.

Discussion

This study represents the first detailed assessment of the presence and pathogenicity of Botryosphaeriaceae and *Phaeoacremonium* species on *C. sempervirens*. Four *Phaeoacremonium* species were found, among which *Pm. parasiticum* comprised 72% of the *Phaeoacremonium* isolates obtained. These species have also been isolated from a number of woody hosts worldwide (Table 3), especially grapevine with diseases such as esca and Petri disease (Pascoe *et al.*, 2004; Mostert *et al.*, 2006). Species of *Phaeoacremoni-*

um are known to cause dieback or decline symptoms on various economically important crops including date palms (Hawksworth *et al.*, 1976), *Prunus* species (Hawksworth *et al.*, 1976; Rumbos, 1986; Damm *et al.*, 2008), kiwifruit (Di Marco *et al.*, 2000), olive trees (Hawksworth *et al.*, 1976) and almond (Gramaje *et al.*, 2012). Of the different *Phaeoacremonium* species, only *Pm. novae-zealandiae* and *Pm. parasiticum* have been reported from *Cupressus* sp. (Mostert *et al.*, 2006). We isolated *Pm. parasiticum* from different types of lesions, including brown to black spots, brown to black streaking, brown internal necroses, watery necroses, dark brown wood discolorations around the canker tissues and wedge-shaped necroses. Similar internal symptoms, including black spots, brown to black streaks, brown internal necroses, central necroses and wedge-shaped necroses have been identified previously from grapevine in Iran (Mohammadi *et al.*, 2013a, 2013b). Six different types of symptoms, including brown streaking, black streaking, wedge-shaped necrosis, watery necrosis, brown internal necrosis and soft rot have been identified associated with trunk diseases in grapevine (Van Niekerk *et al.*, 2011). Similar symptoms have also been reported in pear and apple by Cloete *et al.* (2011). *Phaeoacremonium aleophilum* is known as the most common species on grapevines worldwide (Mostert *et al.*, 2006; Essakhi *et al.*, 2008). In the present study, the less known *Pm. iranianum* was isolated three times from a cypress tree showing dieback in Kerman with symptoms of black spots and brown streaking. Only one isolate of *Pm. rubrigenum* was found on *C. sempervirens* during this study. This species has been associated with human infections (Guarro *et al.*, 2003; Mostert *et al.*, 2005) but it has also been isolated from the galleries and larvae of *Scolytus intricatus* (on *Quercus robur*) and adults of *Leperisinus fraxini* (on *Fraxinus excelsior*) (Kubátová *et al.*, 2004). Essakhi *et al.* (2008) isolated this species from diseased grapevines showing esca symptoms.

Two species of the Botryosphaeriaceae, *B. dothidea* and *N. parvum*, were obtained from cypresses showing canker and decline symptoms and different internal symptoms, including brown internal necrosis, brown to black streaking and wedge-shaped necrosis. Wedge-shaped wood discoloration is commonly associated with the presence of *Botryosphaeria* spp. on grapevine (Castillo-Pando *et al.*, 2001; Phillips, 2002; Savocchia *et al.*, 2007). During the present study, two isolates of *N. parvum* and one of *B. dothidea* were iso-

Table 3. Host plants and worldwide distribution of three *Phaeoacremonium* and two Botryosphaeriaceae species associated with cypress trees.

Fungus	Host plant	Country	Reference
<i>Phaeoacremonium aleophilum</i>	<i>Prunus pennsylvanica</i>	Canada	Hausner <i>et al.</i> (1992)
	<i>Prunus armeniaca</i>	South Africa	Mostert <i>et al.</i> (2006), Damm <i>et al.</i> (2008)
	<i>Prunus salicina</i>	South Africa	Damm <i>et al.</i> (2008)
	<i>Prunus persica</i>	South Africa	Damm <i>et al.</i> (2008)
	<i>Actinidia chinensis</i>	Italy	Groenewald <i>et al.</i> (2001)
	<i>Actinidia deliciosa</i>	Italy	Di Marco <i>et al.</i> (2000)
	<i>Olea europaea</i>	Italy	Groenewald <i>et al.</i> (2001)
	<i>Malus</i> sp.	South Africa	Cloete <i>et al.</i> (2011)
	<i>Vitis vinifera</i>	Worldwide	Mostert <i>et al.</i> (2006)
	<i>Salix</i> sp.	–	Mostert <i>et al.</i> (2006)
<i>Phaeoacremonium parasiticum</i>	<i>Prunus armeniaca</i>	Tunisia	Hawksworth <i>et al.</i> (1976)
	<i>Prunus avium</i>	Greece	Rumbos (1986)
	<i>Phoenix dactylifera</i>	Iraq	Mosteret <i>et al.</i> (2005)
	<i>Prunus avium</i>	Greece	Rumbos (1986)
	<i>Prunus armeniaca</i>	South Africa	Damm <i>et al.</i> (2008)
	<i>Actinidia chinensis</i>	Italy	Groenewald <i>et al.</i> (2001)
	<i>Vitis vinifera</i>	Argentina, Spain, Iran	Dupont <i>et al.</i> (2002), Aroca <i>et al.</i> (2010), Mohammadi <i>et al.</i> (2013a)
	<i>Cupressus</i> sp.	–	Mostert <i>et al.</i> (2006)
<i>Phaeoacremonium iranianum</i>	<i>Actinidia deliciosa</i>	Italy	Mostert <i>et al.</i> (2006)
	<i>Vitis vinifera</i>	Iran, Italy, Spain, South Africa	Mostert <i>et al.</i> (2006), Essakhi <i>et al.</i> (2008), Gramaje <i>et al.</i> (2009b), White <i>et al.</i> (2011)
	<i>Prunus dulcis</i>	Spain	Gramaje <i>et al.</i> (2012)
	<i>Prunus armeniaca</i>	South Africa	Damm <i>et al.</i> (2008)
	<i>Pyrus</i> sp.	South Africa	Cloete <i>et al.</i> (2011)
<i>Neofusicoccum parvum</i>	<i>Acer pseudoplatanus</i>	Italy	Moricca <i>et al.</i> (2012)
	<i>Quercus robur</i>	Italy	Moricca <i>et al.</i> (2012)
	<i>Eucalyptus</i> sp.	Spain, South Africa	Smith <i>et al.</i> (1996), Iturrutxa <i>et al.</i> (2011)
	<i>Vaccinium</i> spp.	Chile, New Zealand	Espinoza <i>et al.</i> (2009), Sammonds <i>et al.</i> (2009)
	<i>Tibouchina</i> spp.	South Africa, New Zealand, Australia	Heath <i>et al.</i> (2011)
	<i>Araucaria heterophylla</i>	Australia	Golzar and Burgess (2011)

(Continued)

Table 3. (Continued)

Fungus	Host plant	Country	Reference
	<i>Cupressus funebris</i>	China	Li <i>et al.</i> (2010)
	<i>Juglans regia</i>	Spain	Moral <i>et al.</i> (2010)
	<i>Vitis vinifera</i>	Spain, New Zealand, USA, Iran	Bonfiglioli and McGregor (2006), Armengol <i>et al.</i> (2001), Úrbez-Torres and Gubler (2009), Mohammadi <i>et al.</i> (2013b)
	<i>Olea europaea</i>	Italy	Lazzizzera <i>et al.</i> (2008)
	<i>Prunus dulcis</i>	Spain	Gramaje <i>et al.</i> (2012)
	<i>Prunus persica</i>	Greece	Thomidis <i>et al.</i> (2011)
	<i>Actinidia deliciosa</i>	New Zealand	Pennycook and Samuels (1985)
	<i>Mangifera indica</i>	Brazil	de Oliveira <i>et al.</i> (2010)
<i>Botryosphaeria dothidea</i>	<i>Vitis vinifera</i>	Portugal, Spain, Iran	Phillips (2002), Aroca <i>et al.</i> (2010), Mohammadi <i>et al.</i> (2013b)
	<i>Ceratonia siliqua</i>	Italy	Granata <i>et al.</i> (2011)
	<i>Pinus</i> spp.	South Africa	Smith <i>et al.</i> (1996)
	<i>Eucalyptus</i> sp.	South Africa	Smith <i>et al.</i> (1996)
	<i>Olea europaea</i>	Greece	Phillips <i>et al.</i> (2005)
	<i>Pistacia vera</i>	USA	Ma <i>et al.</i> (2001)
	<i>Prunus dulcis</i>	USA, Spain	Inderbitzin <i>et al.</i> (2010), Gramaje <i>et al.</i> (2012)
	<i>Cistus ladanifer</i>	Spain	Sánchez-Hernández <i>et al.</i> (2002)
	<i>Actinidia deliciosa</i>	Greece	Thomidis and Exadaktylou (2010)

lated from dark stripes on the wood surface just below the bark and around the cankers from affected trees. Similar symptoms were described by Larignon and Dubos (2001) as characteristic of the black dead arm disease of grapevine. *Neofusicoccum parvum* is one of the most virulent Botryosphaeriaceae species on grapevine worldwide (Phillips, 2002; Van Niekerk *et al.*, 2004; Úrbez-Torres and Gubler, 2009). This species, associated with *Diplodia seriata* De Not., has been isolated from grapevine in Iran (Mohammadi *et al.*, 2013b). *Botryosphaeria dothidea* causes canker diseases in a broad range of woody plants, including several *Prunus* spp. (English *et al.*, 1966).

Recently, this species has been isolated from grapevines showing decline symptoms in Iran (Kerman province) (Arabnezhad and Mohammadi, 2012). *Botryosphaeria dothidea* and *N. parvum* have been isolated and reported from different hosts and geographical locations (Table 3). Among different hosts affected by Botryosphaeriaceae, grapevine is most prominent, but species of this fungus family generally have the ability to colonise a wide range of woody hosts and have been implicated in the decline of different trees. Azouaoui-Idjer *et al.* (2012) isolated and reported *Botryosphaeria iberica* from Monterey cypress (*Cupressus macrocarpa*) showing dieback and mortality in Al-

geria. In Tunisia, *Diplodia pinea* f. sp. *cupressi* has been isolated and reported from Mediterranean cypress showing decline symptoms (Intini *et al.*, 2005). Based on our pathogenicity tests, the lesions caused by *N. parvum* on cypress and grapevine cuttings were larger than those caused by other species. Amponsah *et al.* (2009) found *Botryosphaeria* species, including *B. parva*, on infected grapevines and other woody hosts to produce symptomatic infections on green shoots of grapevine. Li *et al.* (2010) showed *N. parvum* to be pathogenic, causing dark vascular stem tissue on Chinese weeping cypress.

Of the four *Phaeoacremonium* species found during the present study, *Pm. aleophilum* caused the largest lesions on cypress and green shoots of grapevine. This species is one of the main pathogens involved in the esca and Petri disease complex (Mugnai *et al.*, 1999; Mostert *et al.*, 2006). Although *Pm. rubrigenum* failed to produce larger lesions on cypress than controls, the fungus can be considered pathogenic on green shoots of grapevine, in which lesion sizes were larger than in the negative controls. Recently some grapevine trunk pathogens have been isolated from apple and pear trees in South Africa (Cloete *et al.*, 2011). According to that study, *Neofusicoccum australe*, *D. seriata* and *Pm. mortoniae* from infected pear and apple trees, were pathogenic and produced larger lesions on grapevine shoots than the negative controls.

In inoculation tests, *B. dothidea* was pathogenic on green shoots of grapevine and cypress stems. On cypress, *B. dothidea* produced small rounded to elongated lesions and no significant differences could be found between lesions produced by *B. dothidea* and *Pm. iranianum* on this host (Table 2). In Iran, *Pm. aleophilum*, *Pm. parasiticum*, *Pm. iranianum*, *Pm. viticola*, *Pm. inflatipes*, *Pm. cinereum*, *Pm. tuscanum* and *Pm. mortoniae* have previously been isolated and reported from grapevine (Mostert *et al.*, 2006; Gramaje *et al.*, 2009a; Mohammadi, 2012; Mohammadi and Banihashemi, 2012; Mohammadi *et al.*, 2013a). *Phaeoacremonium rubrigenum* has not yet been found on grapevines in Iran, but recently this fungus has been isolated and reported from persimmon (*Diospyros kaki*) trees showing decline symptoms in Shiraz (Fars province, south-western Iran) (Jamali and Banihashemi, 2012). Occurrence of the fungus on grapevine in this country can be expected. The fact that *Phaeoacremonium* and *Botryosphaeriaceae* species associated with diseased cypress trees were also able

to induce typical wood discoloration symptoms on green shoots of grapevine points to the need of more investigations on cypress trees, especially those planted as windbreaks of vineyards (or close to vineyards). It is likely that these trees could be sources of grapevine trunk pathogens.

Based on literature reviews, this is the first report of *B. dothidea*, *N. parvum*, *Pm. aleophilum*, *Pm. rubrigenum* and *Pm. iranianum* and their pathogenicity on *C. sempervirens*.

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Literature cited

- Abdollahzadeh J., E. Mohammadi Goltapeh, A. Javadi, M. Shams-bakhsh, R. Zare and A.J.L. Phillips, 2009. *Barriopsis iraniana* and *Phaeobotryon cupressi*: two new species of the Botryosphaeriaceae from trees in Iran. *Persoonia* 23, 1–8.
- Alves A., A. Correia and A.J.L. Phillips, 2006. Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. *Fungal Diversity* 23, 1–15.
- Amponsah N.T., E.E. Jones, H.J. Ridgway and M.V. Jaspers, 2009. First report of *Neofusicoccum australe* (*Botryosphaeria australis*), a cause of grapevine dieback in New Zealand. *Australasian Plant Disease Notes* 4, 6–8.
- Arabnezhad M. and H. Mohammadi, 2012. Study of esca and Petri disease of grapevine in Kerman province. *Iranian Journal of Plant Pathology* 48, 277–283.
- Armengol J., A. Vicent, L. Torné, F. García-Figueres and J. García-Jiménez, 2001. Fungi associated with esca and grapevine declines in Spain: a three-year survey. *Phytopathologia Mediterranea* 40, S325–S329.
- Aroca A., D. Gramaje, J. Armengol, J. García-Jiménez and R. Raposo, 2010. Evaluation of grapevine nursery process as a source of *Phaeoacremonium* spp. and *Phaeoconiella chlamydozpora* and occurrence of trunk disease pathogens in rootstock mother vines in Spain. *European Journal of Plant Pathology* 126, 165–174.
- Azouaoui-Idjer G., G. Della Rocca, A. Pecchioli, Z. Bouznad and R. Danti, 2012. First report of *Botryosphaeria iberica* associated with dieback and tree mortality of Monterey cypress (*Cupressus macrocarpa*) in Algeria. *Plant Disease* 96, 1073.
- Bagnoli F., G.G. Vendramin, A. Buonamici, A.G. Doulis, S.C. Gonzalez-Martinez, N. La Porta, D. Magri, P. Raddi, F. Sebastiani and S. Fineschi, 2009. Is *Cupressus sempervirens* native to Italy? An answer from genetic and paleobotanical data. *Molecular Ecology* 18, 2267–2286.

- Berraf-Tebbal A., Z. Bouznad, J.M. Santos, M.A. Coelho, J.P. Peros and A.J.L. Phillips, 2011. *Phaeoacremonium* species associated with Eutypa dieback and esca of grapevines in Algeria. *Phytopathologia Mediterranea* 50, 86–97.
- Boesewinkel H.J., 1983. New records of the three fungi causing cypress canker in New Zealand, *Seiridium cupressi* (Guba) comb. nov. and *S. cardinale* on *Cupressocyparis* and *S. unicorn* on *Cryptomeria* and *Cupressus*. *Transactions of the British Mycological Society* 80, 544–547.
- Bonfiglioli R. and S. McGregor, 2006. The *Botryosphaeria* conundrum – a New Zealand perspective. *The Australian & New Zealand Grapegrower & Winemaker* 512, 49–53.
- Bruck R.I., Z. Solfi, I.S. Ben-Ze'ev and A. Zehavi, 1990. Diseases of Italian cypress caused by *Botryodiplodia theobromae* Pat. *European Journal of Forest Pathology* 20, 392–396.
- Castillo-Pando M., A. Sommers, C.D. Green, M. Priest and M. Sriskanthades, 2001. Fungi associated with dieback of Semillon grapevines in the Hunter Valley of New South Wales. *Australasian Plant Pathology* 30, 59–63.
- Cloete M., P.H. Fourie, U. Damm, P.W. Crous and L. Mostert, 2011. Fungi associated with dieback symptoms of apple and pear trees with a special reference to grapevine trunk disease pathogens. *Phytopathologia Mediterranea* 50, 176–190.
- Damm U., L. Mostert, P.W. Crous and P.H. Fourie, 2008. Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. *Persoonia* 20, 87–102.
- De Oliveira Costa V.S., S.J. Michereff, R.B. Martins, M.P.S. Câmara, C.A.T. Gava and E.S.G. Mizubuti, 2010. Species of Botryosphaeriaceae associated on mango in Brazil. *European Journal of Plant Pathology* 127, 509–519.
- Di Marco S., F. Calzarano, W. Gams and A. Cesari, 2000. A new wood decay of kiwifruit in Italy. *New Zealand Journal of Crop and Horticultural Science* 28, 69–73.
- Dupont J., S. Magnin, C. De'sari and M. Gatica, 2002. ITS and β -tubulin markers help delineate *Phaeoacremonium* species, and the occurrence of *Pm. parasiticum* in grapevine disease in Argentina. *Mycological Research* 106, 1143–1150.
- English H., J.R. Davis and J.E. deVay, 1966. Dothiorella canker, a new disease of almond trees in California. *Phytopathology* 56, 146.
- Espinoza J.G., E.X. Briceño, E.R. Chávez, J.R. Úrbez-Torres and B.A. Latorre, 2009. *Neofusicoccum* spp. associated with stem canker and dieback of blueberry in Chile. *Plant Disease* 93, 1187–1194.
- Essakhi S., L. Mugnai, P.W. Crous, J.Z. Groenewald and G. Surico, 2008. Molecular and phenotypic characterization of novel *Phaeoacremonium* species isolated from esca diseased grapevines. *Persoonia* 21, 119–134.
- Gallis A.T., A.G. Doulis and A.C. Papageorgiou, 2006. Variability of cortex terpene composition in *Cupressus sempervirens* L. provenances grown in Crete, Greece. *Silvae Genetica* 56, 294–299.
- Giovanelli A. and A. De Carlo, 2007. Micropropagation of Mediterranean cypress (*Cupressus sempervirens* L.). In: *Protocols for Micropropagation of Woody Trees and Fruits*. (S.M. Jain, H. Häggman, ed.). Springer-Verlag, Dordrecht, The Netherlands, pp. 93–105.
- 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 Biology* 61, 1323–1330.
- Golzar H. and T.I. Burgess, 2011. *Neofusicoccum parvum*, a causal agent associated with cankers and decline of Norfolk Island pine in Australia. *Australasian Plant Pathology* 40, 484–789.
- Gramaje D., J. Armengol, H. Mohammadi, Z. Banihashemi and L. Mostert, 2009a. Novel *Phaeoacremonium* species associated with Petri disease and esca of grapevine in Iran and Spain. *Mycologia* 101, 920–929.
- Gramaje D., J. Armengol, M. Colino, R. Santiago, E. Moralejo, D. Olmo, J. Luque and L. Mostert, 2009b. First report of *Phaeoacremonium inflatipes*, *P. iranianum* and *P. sicilianum* causing Petri disease of grapevine in Spain. *Plant Disease* 93, 964.
- Gramaje D., C. Agustí-Brisach, A. Pérez-Sierra, E. Moralejo, D. Olmo, L. Mostert, U. Damm and J. Armengol, 2012. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). *Persoonia* 28, 1–13.
- Gramaje D., J. Armengol and H.J. Ridgway, 2013. Phenotypic, genetic and virulence diversity, and mating type distribution of *Togninia minima* in Spain. *European Journal of Plant Pathology* (in press).
- Granata G., R. Faedda and A. Sidoti, 2011. First report of canker disease caused by *Diplodia olivarum* on carob tree in Italy. *Plant Disease* 95, 776.
- Graniti A., 1998. Cypress canker: a pandemic in progress. *Annual Review of Phytopathology* 36, 91–114.
- Groenewald M., J.C. Kang and P.W. Crous, 2001. ITS and beta-tubulin phylogeny of *Phaeoacremonium* and *Phaeomoniella* species. *Mycological Research* 105, 651–657.
- Guarro J., S.H. Alves, J. Gené, N.A. Grazziotin, R. Mazzuco, C. Dalmagro, J. Capilla, L. Zaror and E. Mayayo, 2003. Two cases of subcutaneous infection due to *Phaeoacremonium* spp. *Journal of Clinical Microbiology* 41, 1332–1336.
- Hausner G., G.G. Eyjólfsson, J. Reid and G.R. Klassen, 1992. Two additional species of the genus *Togninia*. *Canadian Journal of Botany* 70, 724–732.
- Hawksworth D.L., I.A.S. Gibson and W. Gams, 1976. *Phialophora parasitica* associated with disease conditions in various trees. *Transactions of British Mycological Society* 66, 427–431.
- Heath R.N., J. Roux, B. Slippers, A. Drenth, S.R. Pennycook, B.D. Wingfield and M.J. Wingfield, 2011. Occurrence and pathogenicity of *Neofusicoccum parvum* and *N. mangiferae* on ornamental *Tibouchina* species. *Forest Pathology* 41, 48–51.
- Inderbitzin P., R.M. Bostock, F.P. Trouillas and T.J. Michailides, 2010. A six locus phylogeny reveals high species diversity in Botryosphaeriaceae from California almond. *Mycologia* 102, 1350–1368.
- Intini M., A. Panconesi, G. Stanosz and D. Smith, 2005. First report of Diplodia canker of cypress caused by *Diplodia pinea* f. sp. *cupressi* on Mediterranean cypress in Tunisia. *Disease Notes* 89, 1246.
- Iturriza E., B. Slippers, N. Mesanza and M.J. Wingfield, 2011. First report of *Neofusicoccum parvum* causing canker and die-back of *Eucalyptus* in Spain. *Australasian Plant Disease Notes* 6, 57–59.

- Jamali S. and Z. Banihashemi, 2012. First report of *Phaeoacremonium rubrigenum*, associated with declining persimmon trees in Iran. *Journal of Plant Protection* 1, 153–159.
- Jurc D., N. Ogris, T. Grebenc and H. Kraigher, 2006. First report of *Botryosphaeria dothidea* causing bark dieback of European hop hornbeam in Slovenia. *Plant Pathology* 55, 299.
- Kubátová A., M. Kolařík and S. Pažoutová, 2004. *Phaeoacremonium rubrigenum* – hyphomycete associated with bark beetles found in Czechia. *Folia Microbiologica* 49, 99–104.
- Larignon P. and B. Dubos, 2001. The villainy of black dead arm. *Wines Vines* 82, 86–89.
- Lazzizzera C., S. Frisullo, A. Alves and A.J.L. Phillips, 2008. Morphology, phylogeny and pathogenicity of *Botryosphaeria* and *Neofusicoccum* species associated with drupe rot of olives in southern Italy. *Plant Pathology* 57, 948–956.
- Li S.B., J.Z. Li, S.C. Li, Z.H. Lu, J.H. Wang and H. Zhang, 2010. First report of *Neofusicoccum parvum* causing dieback disease of Chinese weeping cypress in China. *Disease Notes* 94, 641.
- Linde C., G.H.J. Kemp and M.J. Wingfield, 1997. First report of Sphaeropsis canker of cypress in South Africa. *European Journal of Forest Pathology* 27, 173–177.
- Ma Z., E.W.A. Boehm, Y. Luo and T.J. Michailides, 2001. Population structure of *Botryosphaeria dothidea* from pistachio and other hosts in California. *Phytopathology* 91, 665–672.
- Madar Z., Z. Solel and M. Kimcii, 1991. Pestalotiopsis canker of cypress in Israel. *Phytoparasitica* 19, 79–81.
- Martos S., E. Torres, M. Abdessamad El Bakali, R. Raposo, D. Gramaje, J. Armengol and J. Luque, 2011. Co-operational PCR coupled with dot blot hybridization for the detection of *Phaeoconiella chlamydospora* on infected grapevine wood. *Journal of Phytopathology* 159, 247–254.
- Mohammadi H., 2012. First report of *Phaeoacremonium tuscanum* causing Petri disease of grapevine in Iran. *New Disease Reports* 25, 21.
- Mohammadi H. and Z. Banihashemi, 2012. First report of *Phaeoacremonium inflatipes* and *Phaeoacremonium mortoniae* associated with grapevine Petri disease in Iran. *Journal of Agricultural Science and Technology* 14, 1405–1414.
- Mohammadi H., Z. Banihashemi, D. Gramaje and J. Armengol, 2013a. Fungal pathogens associated with grapevine trunk diseases in Iran. *Journal of Agricultural Science and Technology* 15, 137–150.
- Mohammadi H., Z. Banihashemi, D. Gramaje and J. Armengol, 2013b. Characterization of *Diplodia seriata* and *Neofusicoccum parvum* associated with grapevine decline in Iran. *Journal of Agricultural Science and Technology* 15, 603–616.
- Moral J., C. Muñoz-Díez, N. González, A. Trapero and T.J. Michailides, 2010. Characterization and pathogenicity of *Botryosphaeriaceae* species collected from olives and other hosts in Spain and California. *Phytopathology* 100, 1340–1351.
- Moricca S., A. Uccello, B. Ginetti and A. Ragazzi, 2012. First report of *Neofusicoccum parvum* associated with bark canker and dieback of *Acer pseudoplatanus* and *Quercus robur* in Italy. *Plant Disease* 96, 1699.
- Mostert L., J.Z. Groenewald, R.C. Summerbell, V. Robert, D.A. Sutton, A.A. Padhye and P.W. Crous, 2005. Species of *Phaeoacremonium* associated with infections in humans and environmental reservoirs in infected woody plants. *Journal of Clinical Microbiology* 43, 1752–1767.
- Mostert L., J.Z. Groenewald, R.C. Summerbell, W. Gams and P.W. Crous, 2006. Taxonomy and pathology of *Togninia* (Diaporthales) and its *Phaeoacremonium* anamorphs. *Studies in Mycology* 54, 115 pp.
- Mugnai L., A. Graniti and G. Surico, 1999. Esca (black meales) and brown wood-streaking: two old and elusive diseases of grapevines. *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.
- Panconesi A., 1990. Pathological disorders in the Mediterranean basin. In: *Agrimed Research Programme, Progress in EEC Research on Cypress Diseases* (J. Ponchet, ed.). Report EUR 12493 EN, Luxembourg, pp. 54–81.
- Pascoe I.G., J. Edwards, J.H. Cunnington and E.H. Cottral, 2004. Detection of the *Togninia* teleomorph of *Phaeoacremonium aleophilum* in Australia. *Phytopathologia Mediterranea* 43, 51–58.
- Pennycook S.R. and G.J. Samuels, 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. *Mycotaxon* 24, 445–458.
- Phillips A., 2002. *Botryosphaeria* species associated with diseases of grapevine in Portugal. *Phytopathologia Mediterranea* 41, 3–18.
- Phillips A.J.L., I.C. Rumbos, A. Alves and A. Correia, 2005. Morphology and phylogeny of *Botryosphaeria dothidea* causing fruit rot of olives. *Mycopathologia* 159, 433–439.
- Rumbos I.C., 1986. *Phialophora parasitica*, causal agent of cherry die-back. *Journal of Phytopathology* 117, 283–287.
- Sammonds J., R. Billones, M. Rocchetti, H.J. Ridgway, M. Walter and M.V. Jaspers, 2009. Survey of Blueberry farm for *Botryosphaeria* dieback and crown rot pathogens. *New Zealand Plant Protection* 62, 238–242.
- Sánchez-Hernández M.E., J. Gutiérrez-García and A. Trapero-Casas, 2002. *Botryosphaeria* canker of *Cistus ladanifer*. *Plant Pathology* 51, 365–373.
- Savocchia S., C.C. Steel, B.J. Stodart and A. Somers, 2007. Pathogenicity of *Botryosphaeria* species isolated from declining grapevines in subtropical regions of Eastern Australia. *Vitis* 46, 27–32.
- Smith H., M.J. Wingfield, P.W. Crous and T.A. Coutinho, 1996. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* 62, 86–88.
- Solel Z., R. Messinger, Y. Golan and Z. Madar, 1983. Coryneum canker of cypress in Israel. *Plant Disease* 67, 550–551.
- Solel Z., Z. Madar, M. Kimchi and Y. Golan, 1987. *Diplodia* canker of cypress. *Canadian Journal of Plant Pathology* 9, 115–118.
- Thomidis T. and E. Exadaktylou, 2010. First report of *Botryosphaeria dothidea* causing shoot blight of kiwifruit (*Actinidia deliciosa*) in Greece. *Plant Disease* 94, 1503.
- Thomidis T., T.J. Michailides and Exadaktylou E., 2011. *Neofusicoccum parvum* associated with fruit rot and shoot blight of peaches in Greece. *European Journal of Plant Pathology* 131, 661–668.

- Úrbez-Torres J.R. and W.D. Gubler, 2009. Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California. *Plant Disease* 93, 584–92.
- Úrbez-Torres J., G. Leavitt, T. Voegel and W.D. Gubler, 2008. Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the causal agents of bot canker disease of grapevines in Mexico. *Plant Disease* 92, 519–529.
- Van der Werff H.S., 1988. Cypress canker in New Zealand plantations. *New Zealand Journal of Forestry Science* 18, 101–108.
- Van Niekerk J.M., W. Bester, F. Halleen, P.W. Crous and P.H. Fourie, 2011. The distribution and symptomatology of grapevine trunk disease pathogens are influenced by climate. *Phytopathologia Mediterranea* 50, S98–S111.
- Van Niekerk J.M., P.W. Crous, J.Z. Groenewald, P.H. Fourie and F. Halleen, 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevine. *Mycologia* 96, 781–798.
- White T.J., T. Bruns, J. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a Guide to Methods and Applications*. (Innis M.A., Gelfand D.H., Sninsky J.J., White T.J, eds.), Academic Press, San Diego, CA, USA, pp. 315–322.
- White C.L., F. Halleen, M. Fischer and L. Mostert, 2011. Characterisation of the fungi associated with esca diseased grapevines in South Africa. *Phytopathologia Mediterranea* 50, 204–223.
- Zohary M., 1973. *Geobotanical Foundation of the Middle East*. Gustav Fischer Verlag, Stuttgart, Germany, 738 pp.

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