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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 (Giovanelli 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* (PMPCYP1, 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-

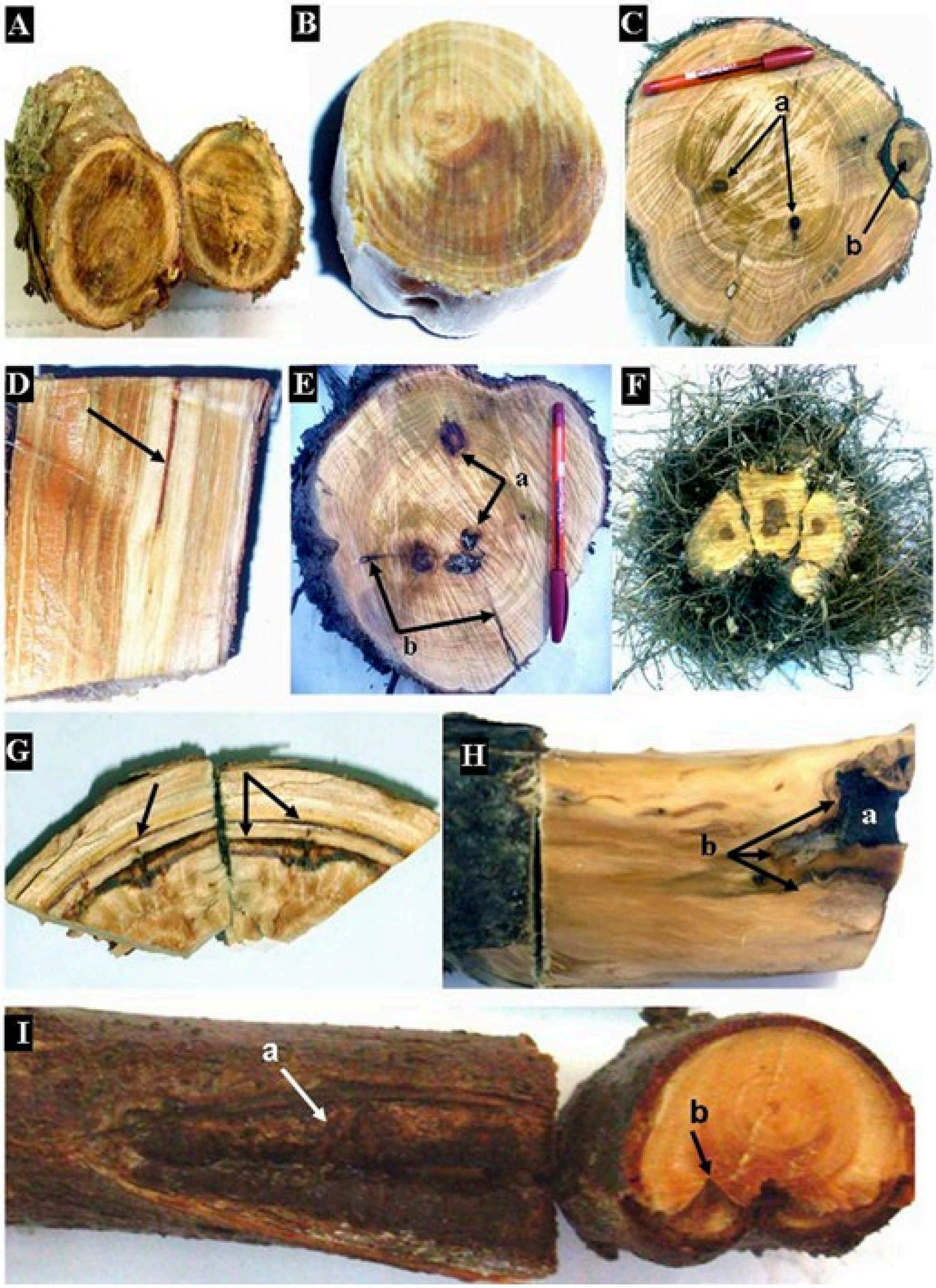


**Figure 1.** Symptoms associated with trunk disease on cypress trees. A, central wood necrosis. B, watery necrosis, C, co-occurrence of black spots (a) and central necrosis on a lateral branch (b). D, vascular discoloration as a brown line in longitudinal section, indicated by arrow. E, co-occurrence 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 | |
| *Phaeoacremonium parasiticum* | 52 (30.4%) | + | + | + | Y,DI,D,W | 22 | 12 | 8 | 7 | 1 | 2 | Kerman, Mahan, Jiroft, Sirch, Ravar, Pariz ,Sirjan |
| *Phaeoacremonium aleophilum* | 16 (9.4%) | + | + | + | Y,DI,D | 11 | 3 | 2 | – | – | – | Sirjan, Mahan, Sirch, Kerman |
| *Phaeoacremonium iranianum* | 3 (1.7%) | – | + | – | DI | 2 | – | 1 | – | – | – | Kerman |
| *Phaeoacremonium rubrigenum* | 1 (0.6%) | – | – | + | Y | 1 | – | – | – | – | – | Kerman |
| *Neofusicoccum parvum* | 8 (4.7%) | – | – | + | C,D | – | 2 | 1 | – | 2 | 3 | Sirch, Mahan |
| *Botryosphaeria dothidea* | 7 (4.1%) | – | – | + | C, DI | – | 1 | 1 | – | 1 | 4 | Kerman, Sirjan |
| *Paecilomyces variotii* | 18 (10.5%) | – | + | + | Y,DI,D | – | 5 | 8 | – | 5 | – | Kerman, Mahan, Jiroft, |
| *Alternaria* sp. | 9 (5.3%) | + | + | + | Y,D | 6 | – | – | – | 3 | – | Kerman, Mahan, Jiroft |
| *Nattrassia mangiferae* | 11 (6.4%) | – | – | + | DI,D | 3 | 8 | – | – | – | – | Sirjan, Kerman |
| *Fusarium equiseti* | 6 (3.5%) | – | + | – | D,DI | – | 2 | 4 | – | – | – | Kerman |
| *Fusarium* spp. | 15 (8.8%) | + | + | + | Y,DI,D | – | 9 | 6 | – | – | – | Kerman, Jiroft, Sirch, Ravar, ,Sirjan |
| *Aspergillus* sp. | 8 (4.7%) | – | – | – | DI,D | 4 | 3 | 1 | – | – | – | Sirjan, Mahan, Sirch |
| *Penicillium* sp. | 11 (6.4%) | + | – | + | DI,D | 5 | 4 | – | – | 2 | – | Kerman, Mahan, Jiroft, Sirch, Ravar |
| *Phoma* 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 |
| *Phaeoacremonium aleophilum* | KRC-12 | PACYP1 | KC480187 | 20.45 b | 28.88 b | 66.6 | 91.6 |
| *Phaeoacremonium parasiticum* | KRM-8 | PMPCYP1 | KC467058 | 18.50 b | 23.25 c | 75.0 | 83.3 |
| *Phaeoacremonioum iranianum* | KRS-1 | PMICYP1 | KC416211 | 13.20 c | 24.87 c | 41.7 | 33.3 |
| *Phaeoacremonium rubrigenum* | KRK-1 | PRCYP1 | KC416210 | 7.50 d | 10.80 e | 50.0 | 16.7 |
| *Neofusicoccum parvum* | KRM-5 | NPCYP1 | KC467060 | 23.45 a | 34.87 a | 83.3 | 91.6 |
| *Botryosphaeria dothidea* | 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 *Penicillum* 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 *Phaeoacremonium* 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 |
|---|---|---|---|
| *Phaeoacremonium aleophilum* | *Prunus pennsylvanica* | Canada | Hausner *et al*. (1992) |
| | *Prunus armeniaca* | South Africa | Mostert *et al*. (2006), Damm *et al*. (2008) |
| | *Prunus salicina* | South Africa | Damm *et al*. (2008) |
| | *Prunus persica* | South Africa | Damm *et al*. (2008) |
| | *Actinidia chinensis* | Italy | Groenewald *et al*. (2001) |
| | *Actinidia deliciosa* | Italy | Di Marco *et al*. (2000) |
| | *Olea europaea* | Italy | Groenewald *et al*. (2001) |
| | *Malus* sp. | South Africa | Cloete *et al*. (2011) |
| | *Vitis vinifera* | Worldwide | Mostert *et al*. (2006) |
| | *Salix* sp. | – | Mostert *et al*. (2006) |
| *Phaeoacremonium parasiticum* | *Prunus armeniaca* | Tunisia | Hawksworth *et al*. (1976) |
| | *Prunus avium* | Greece | Rumbos (1986) |
| | *Phoenix dactylifera* | Iraq | Mosteret *et al*. (2005) |
| | *Prunus avium* | Greece | Rumbos (1986) |
| | *Prunus armeniaca* | South Africa | Damm *et al*. (2008) |
| | *Actinidia chinensis* | Italy | Groenewald *et al*. (2001) |
| | *Vitis vinifera* | Argentina, Spain, Iran | Dupont *et al*. (2002), Aroca *et al*. (2010), Mohammadi *et al*. (2013a) |
| | *Cupressus* sp. | – | Mostert *et al*. (2006) |
| *Phaeoacremonium iranianum* | *Actinidia deliciosa* | Italy | Mostert *et al*. (2006) |
| | *Vitis vinifera* | Iran, Italy, Spain, South Africa | Mostert *et al*. (2006), Essakhi *et al*. (2008), Gramaje *et al*. (2009b), White *et al*. (2011) |
| | *Prunus dulcis* | Spain | Gramaje *et al*. (2012) |
| | *Prunus armeniaca* | South Africa | Damm *et al*. (2008) |
| | *Pyrus* sp. | South Africa | Cloete *et al*. (2011) |
| *Neofusicoccum parvum* | *Acer pseudoplatanus* | Italy | Moricca *et al*. (2012) |
| | *Quercus robur* | Italy | Moricca *et al*. (2012) |
| | *Eucalyptus* sp. | Spain, South Africa | Smith *et al*. (1996), Iturritxa *et al*. (2011) |
| | *Vaccinium* spp. | Chile, New Zealand | Espinoza *et al*. (2009), Sammonds *et al*. (2009) |
| | *Tibouchina* spp. | South Africa, New Zealand, Australia | Heath *et al*. (2011) |
| | *Araucaria heterophylla* | Australia | Golzar and Burgess (2011) |

(Continued)

**Table 3.** (Continued)

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