PHAEOACREMONIUM SPECIES ASSOCIATED WITH NECROTIC WOOD OF POME FRUIT TREES IN IRAN

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SUMMARY

During 2011–2013 various pome fruit tree orchards were inspected to study the fungi associated with trunk diseases in the Kerman and Fars provinces of Iran. Wood samples were collected from branches of apple, pear, quince and hawthorn trees showing yellowing, wilting, dieback, cankers and various internal wood discolorations. Six species of *Phaeoacremonium* namely, *Pm. aleophilum*, *Pm. parasiticum*, *Pm. rubrigenum*, *Pm. scolyti*, *Pm. mortoniae* and *Pm. iranianum* were identified based on morphology, culture characteristics and the partial sequences of the β-tubulin gene (BT) data. *Pm. aleophilum* (16.1% of total isolates) and *Pm. parasiticum* (9.8% of total isolates) were the species most frequently isolated. We report *Phaeoacremonium* spp. on quince and hawthorn for the first time in the world. This is also the first report of isolation of *Pm. parasiticum*, *Pm. iranianum* and *Pm. mortoniae* from apple wood, while *Pm. aleophilum*, *Pm. rubrigenum*, *Pm. scolyti* and *Pm. parasiticum* are newly reported from pear trees. Tests were carreid out to determine the pathogenic role of these species on pome trees and grapevine. On grapevine shoots, *Pm. aleophilum* and *Pm. mortoniae* induced the longest and smallest lesions, respectively. On apple and pear shoots, the most aggressive species was *Pm. iranianum* while *Pm. mortoniae* was the least aggressive based on the length of vascular discoloration. On quince, the longest and smallest lesions were caused by *Pm. iranianum* and *Pm. mortoniae*, respectively, but the lesion lengths of *Pm. mortoniae* were also significantly different from the negative controls.

Key words: *Phaeoacremonium*, wood discoloration, trunk diseases, pome fruits, β-tubulin.

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INTRODUCTION

The genus *Phaeoacremonium* W. Gams, Crous et M.J. Wingf. was established 18 years ago with six new species. *Phaeoacremonium parasiticum* W. Gams, Crous & M.J. Wingf (formerly known as *Phialophora parasitica* Ajello, Georg et C.J.K. Wang) was designated as the type species of the genus (Crous *et al*., 1996). *Phaeoacremonium* species have been associated with human infections (Ajello *et al*., 1974; Hemashettar *et al*., 2006), as well as diseases of a number of woody hosts worldwide (Rumbos, 1986; Kubátová *et al*., 2004), including the esca and Petri diseases of the grapevine (Rooney-Latham *et al*., 2005; Mostert *et al*., 2006b; Aroca and Raposo, 2007). Currently, 42 *Phaeoacremonium* species are known, 27 of which have been isolated from grapevine and identified based on morphology, cultural characteristics, and phylogeny (Mostert *et al*., 2006a; Graham *et al*., 2009; Gramaje *et al*., 2009; Úrbez-Torres *et al*., 2014). Different *Phaeoacremonium* species have also been isolated from stone and pome fruit trees. In the latter, several fungi belonging to various genera, i.e. *Eutypa* (Glawe *et al*., 1983), *Phomopsis* (Kanematsu, 2002), *Botryosphaeria*, *Schizophyllum*, *Diplodia* (Crous *et al*., 2000) and *Neofusicoccum* (Cloete *et al*., 2011) are known to cause trunk diseases. Four different *Phaeoacremonium* species, namely *Pm. aleophilum* W. Gams, Crous, M.J. Wingf. et Mugnai, *Pm. iranianum* L. Mostert, Graf., W. Gams et Crous, *Pm. mortoniae* Crous et W. Gams and *Pm. viticola* J. Dupont, were recorded from pome fruit trees in South Africa (Cloete *et al*., 2011). *Phaeoacremonium* species that cause grapevine trunk diseases were shown not to be host specific, and have been isolated from other woody hosts such as pome fruit trees (Table 1).

In spring of 2011, a severe dieback of apple trees was observed in some orchards (mixed with grapevines) in Abadeh (Shiraz province, Iran). Wedge shape and circular wood necrosis similar to those occurring in grapevines were observed in cross sections of affected branches. Although nine *Phaeoacremonium* species have been reported on grapevine in Iran, much less is known on their occurrence on fruit trees which, in this country, are often grown in association with grapes. Therefore, the aim of this study was to identify the fungal species associated with pome fruit trees decline in Iran, with specific reference to *Phaeoacremonium* species known to occur on grapes in this country.

Table 1. A list of *Phaeoacremonium* species that have been found on grapevine and pome fruit trees.

Fungus	Host plant	Reference
*Phaeoacremonium aleophilum**	*Malus* sp.	Cloete *et al.* (2011)
	Vitis vinifera	Crous *et al.* (1996); Mohammadi *et al.* (2013)
	Pyrus sp.	Cloete *et al.* (2011)
*Phaeoacremonium parasiticum**	*Vitis vinifera*	Crous *et al.* (1996); Mohammadi *et al.* (2013)
*Phaeoacremonium iranianum**	*Pyrus* sp.	Cloete *et al.* (2011)
	Vitis vinifera	Mostert *et al.* (2006a)
*Phaeoacremonium mortoniae**	*Pyrus* sp.	Cloete *et al.* (2011); Mohammadi and Banihashemi (2012)
	Malus sp.	Rooney-Latham *et al.* (2006)
	Vitis vinifera	Groenewald *et al.* (2001); Mohammadi and Banihashemi (2012)
Phaeoacremonium angustius	*Malus* sp.	Rooney-Latham *et al.* (2006)
	Vitis vinifera	Crous *et al.* (1996)
*Phaeoacremonium viticola**	*Pyrus* sp.	Cloete *et al.* (2011)
	Vitis vinifera	Dupont *et al.* (2000)
Phaeoacremonium krajdenii	*Vitis vinifera*	Mostert *et al.* (2005)
Phaeoacremonium armeniacum	*Vitis vinifera*	Graham *et al.* (2009)
Phaeoacremonium scolyti	*Vitis vinifera*	Mostert *et al.* (2006a)
Phaeoacremonium austroafricanum	*Vitis vinifera*	Mostert *et al.* (2006a)
*Phaeoacremonium cinereum**	*Vitis vinifera*	Gramaje *et al.* (2009)
*Phaeoacremonium tuscanum**	*Vitis vinifera*	Essakhi *et al.* (2008); Mohammadi (2012)
Phaeoacremonium globosum	*Vitis vinifera*	Graham *et al.* (2009)
Phaeoacremonium griseorubrum	*Vitis vinifera*	Mostert *et al.* (2005)
Phaeoacremonium occidentale	*Vitis vinifera*	Graham *et al.* (2009)
Phaeoacremonium hungaricum	*Vitis vinifera*	Essakhi *et al.* (2008)
Phaeoacremonium croatiense	*Vitis vinifera*	Essakhi *et al.* (2008)
Phaeoacremonium australiense	*Vitis vinifera*	Mostert *et al.* (2005)
*Phaeoacremonium rubrigenum**	*Vitis vinifera*	Crous *et al.* (1996); Mohammadi (2013)
*Phaeoacremonium inflatipes**	*Vitis vinifera*	Crous *et al.* (1996); Mohammadi *et al.* (2013)
Phaeoacremonium hispanicum	*Vitis vinifera*	Gramaje *et al.* (2009)
Phaeoacremonium subulatum	*Vitis vinifera*	Mostert *et al.* (2005)
Phaeoacremonium sicilianum	*Vitis vinifera*	Essakhi *et al.* (2008)
Phaeoacremonium venezuelense	*Vitis vinifera*	Mostert *et al.* (2005)
Phaeoacremonium alvesii	*Vitis vinifera*	Mostert *et al.* (2005)
Phaeoacremonium canadense	*Vitis vinifera*	Úrbez-Torres *et al.* (2014)
Phaeoacremonium roseum	*Vitis vinifera*	Úrbez-Torres *et al.* (2014)

*= *Phaeoacremonium* species that have been reported from grapevine in Iran.

MATERIALS AND METHODS

Sampling and fungal isolation. A field survey was conducted on pome fruit trees to identify the fungi associated with trunk diseases in the provinces of Kerman (south-eastern Iran) and Fars (south-western Iran) between April 2011 and July 2013. Wood samples were collected from branches of pear (*Pyrus communis*), apple (*Malus domestica*), quince (*Cydonia oblonga*) and hawthorn (*Crataegus pontica*) trees showing yellowing, wilting, dieback, decline, defoliation, cankers and internal wood necrosis, black vascular streaking or discolored tissues (Fig. 1). Cross and longitudinal sections were made at various places in the branches of each plant to investigate the distribution of internal wood necrosis. For fungal isolations, wood sections with internal necrosis were selected and cut into smaller sections. Pieces of wood measuring *ca.* 3×3×3 mm were excised from the margins between necrotic and healthy tissue. Wood chips were immersed in 1.5% sodium hypochlorite solution for 60 sec, washed three times with sterile distilled water (SDW) and plated onto malt extract agar [MEA (Merck, Germany)] supplemented with 100 mg/l 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, Germany)] or MEA plates. They were single-spored prior to morphological and molecular identification.

Fungal identification. *Morphological and cultural studies.* The initial identification of the isolates was made based on colony morphology according to visual characteristics such as colony colour and growth. *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, Germany)]. Microscopic observations of phialide type and shape, conidiophore morphology and hyphal wart size from aerial mycelium of the colonies were made on MEA. Radial growth of isolates was measured after 16 days at 25°C (Mostert *et al.*, 2006a).

DNA extraction and molecular studies. Species identifications were confirmed by analysis of the partial sequence of the β-tubulin (BT) gene. Fungal isolates selected for DNA extraction were cultured on PDA and incubated at 25°C for 12 days. Fungal mycelium and

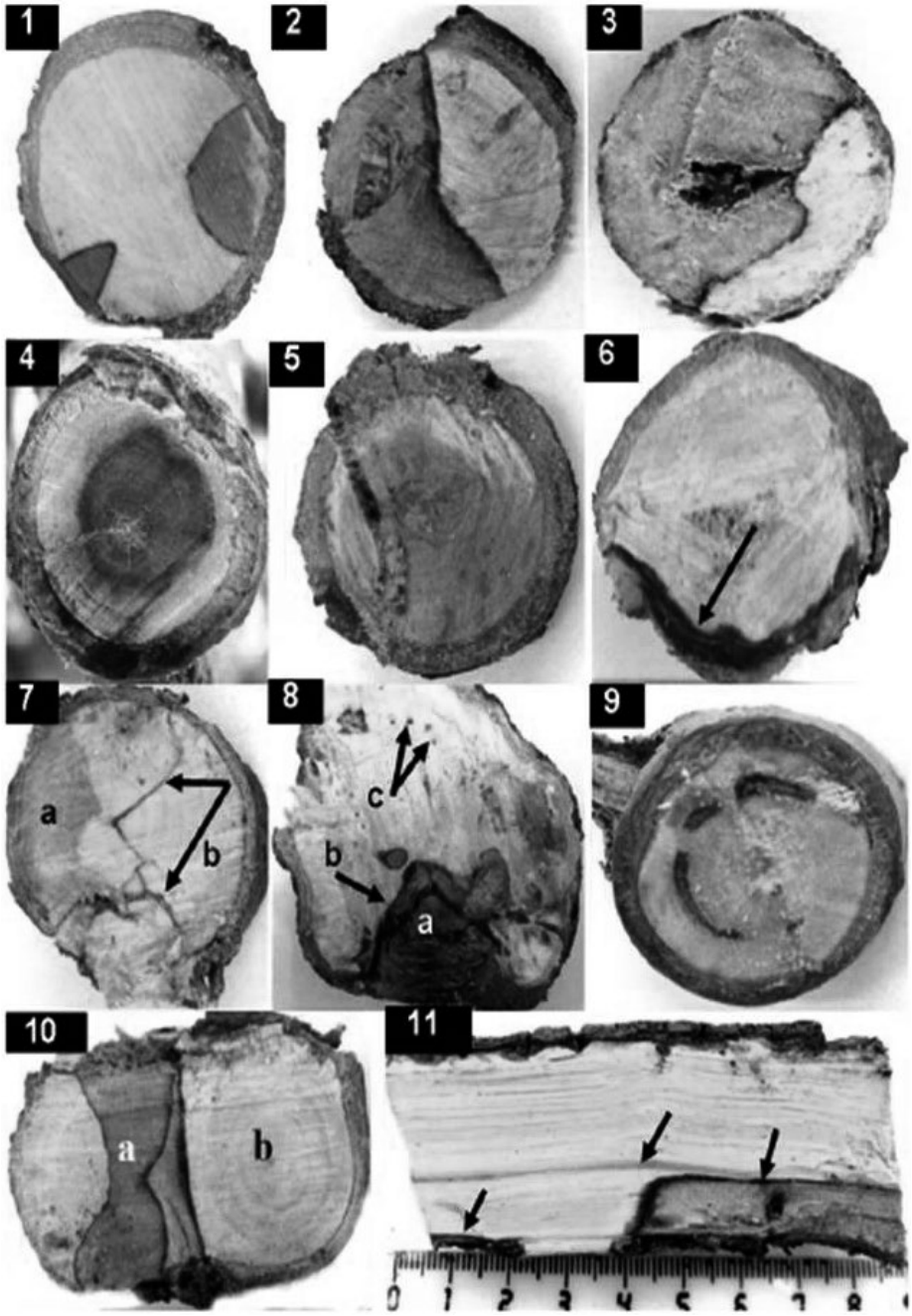

Fig. 1. Various internal symptom types associated with affected pome fruit trees in Iran. 1: Typical wedge-shaped necrosis on quince. 2 and 3: Wood necrosis expanded to occupy around half and even all interior of the affected branchs of apple and quince trees, respectively. 4: Central brown internal necrosis on apple. 5: Watery necrosis on apple. 6: Dark brown wood discoloration just below the bark on hawthorn. 7: Co-occurrence of brown internal necrosis (a) and black wood streaking (b) on quince. 8: Co-occurrence of brown internal necrosis (a), black wood streaking (b) and browon to black spots on quince. 9: Arch shaped necrosis on pear. 10: Central necrosis on a lateral branch (a) compared with a healthy main branch of quince (b). 11: Brown and black line (indicated by arrows) in longitudinal section of an affected branch of quince.

conidia were scraped and mechanically disrupted by grinding to a fine powder in liquid nitrogen with mortar and pestle. Total genomic DNA was extracted with the Peq Gold Fungal DNA mini kit (Roche, Germany) following the manufacturer's instructions. DNA was visualized on 0.1% agarose gels stained with ethidium bromide. DNA samples were kept at –20°C until use for PCR amplifications. The β-tubulin gene was amplified in isolates identified as *Phaeoacremonium* using the primer pair T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995). Each PCR reaction contained 1× PCR buffer, 200 µM each dNTP, 0.5 µM of each primer, 1.5 mM $MgCl_2$, 1.25 U of DNA *Taq* polymerase (Cinnagen, Iran) and 1 µl of template DNA. The PCR reaction mix was adjusted to a final volume of 25 µl with water [Chromasolv Plus (Sigma-Aldrich, Germany)]. PCR amplifications were

performed on a Techne TC-312 Termal Cycler (Techne, UK) with the following program: (i) an initial denaturation step at 94°C for 5 min; (ii) 40 cycles, consisting of denaturation (30 sec at 94°C), annealing (30 sec at 52°C), and extension (50 sec at 72°C); and (iii) a final extension step of 7 min at 72°C. Amplification products were analysed by electrophoresis in 1.5% agarose in TAE buffer, were purified with the High Pure PCR Product Purification Kit (Bioneer, Germany) and custom sequenced (Bioneer Corporation, South Korea).

Pathogenicity tests. Fourteen isolates of *Phaeoacremonium* spp. from different hosts were used for pathogenicity tests. Artificial inoculations were conducted on detached woody shoots (15-20 mm in diameter) of 10-year-old grapevines of cv. Askari, quince (25-year-old), pear (20-year-old) and apple (25-year-old). For each isolate, 8 detached shoots showing neither foliar symptoms or wood discoloration, were collected from each tree species and cut into 35 cm pieces. The outer bark of each sample was cleaned with 70% ethanol at the inoculation site. Shoots were incubated inside a laminar flow cabinet for 1.5 min to dry off the alcohol. A superficial wound (4×4 mm, reaching into the xylem) was made on the shoot of each plant using a 4 mm sterilized cork borer. Shoots were inoculated with a 4-mm-diameter mycelial plug from 16-day-old cultures of each fungus placed on the wounds. Wounds were then sealed with a sheet of Parafilm (Pechiney Plastic Packaging, USA) to prevent desiccation of inoculated site. Control plants were inoculated with sterile PDA plugs. The base of inoculated shoots was inserted into bottles filled with water (about 1000 ml) which were covered with a plastic bag. Inoculated shoots were maintained for 35 days at room temperature (23 to 25°C). The shoots were then collected, the bark of each sample was removed and the extent of vascular discoloration was measured upward and downward from the point of inoculation. 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, USA) was done to evaluate differences in the extent of the lesions induced by fungal isolates for each plant species. The LSD test was used for comparison of treatment means at $P < 0.01$.

RESULTS

Survey and sample collection. A total the 127 samples were collected in the surveyed fields. Of these, 78 were from apple (46), pear (12), quince (18) and howthorn (2) trees from Fars and 49 were from apple (21), pear (9) and quince (19) from Kerman province. Successful fungal isolations were from 92 samples (72.44%) of trees showing outer symptoms such as dieback, chlorosis and wilting of the leaves on some branches, defoliation, cankers and black spots, whereas internal symptoms consisted of wedge-shaped or arch-shaped necrosis, variously extended brown discolorations and brown to black streaking (Fig. 1). In the most severe cases, wood necrosis involved half or even all the interior of the trunk. In some cases, disease symptoms such as dieback, chlorosis and wilting were observed only on one side of the trees and wood discoloration was visible in cross- sections of the affected branches.

Fungal isolation and identification. A total of 193 fungal isolates were recovered from pome trees in Fras and Kerman provinces. Six species of *Phaeoacremonium*: *Pm. aleophilum* (31 isolates), *Pm. parasiticum* (19 isolates), *Pm. iranianum* (8 isolates), *Pm. mortoniae* (5 isolates), *Pm. rubrigenum* (7 isolates) and *Pm. scolyti* (3 isolates), representing 37.82% of the whole isolates (73), were associated with necrotic tissue of pome fruit trees (Table 2). All β-tubulin gene sequences from the above *Phaeoacremonium* isolates had 99-100% homology with those of related species present in GenBank.

Phaeoacremonium aleophilum was the most common species recovered in both provinces, while the other *Phaeoacremonium* species were isolated with a lower frequency: *Pm. parasiticum* (9.8%), *Pm. iranianum* (4.1%), *Pm. rubrigenum* (3.6%) *Pm. mortiniae* (2.6%) and *Pm. scolyti* (1.6%). The association of *Phaeoacremonium* species with the internal symptom types is given in Table 2.

Pm. aleophilum, was isolated 31 times either from apple (14 isolates), pear (7 isolates) and quince (10 isolates) trees showing various outer and internal symptoms such as brown to black spots (5 isolates), brown internal necrosis (8 isolates), brown to black streaking (5 isolates), watery necrosis (4 isolates), dark brown wood discoloration just below the bark (3 isolates), wedge-shaped necrosis (3 isolates) and arch- shaped necrosis (3 isolates).

Nineteen isolates of *Pm. parasiticum* were obtained from apple (6 isolates), pear (2 isolates) and quince (11 isolates) trees. With the exception of watery necrosis, this species was also recovered from other lesion types including brown to black spots (2 isolates), brown internal necrosis (4 isolates), brown to black streaking (3 isolates), dark brown wood discoloration just below the bark (2 isolates), wedge-shaped necrosis (7 isolates) and arch-shaped necrosis (one isolate).

Eight isolates of *Pm. iranianum* came from apple (one isolates), quince (4 isolates) and hawthorn (3 isolates) trees showing brown internal necrosis (5 isolates) and watery necrosis (3 isolates).

Pm. rubrigenum (7 isolates) and *Pm. scolyti* (3 isolates) were isolated from pear and quince in Abadeh (Fars province) and Bam (Kerman province). In particular, *Pm. rubrigenum* was obtained from branches showing brown to black streaking (4 isolates) and wedge-shaped necrosis (3 isolates), while *Pm. scolyti* was recovered only from wedge-shaped necrosis.

Five isolates of *Pm. mortoniae* came from apple trees showing dieback and brown internal necrosis (4 isolates) and brown to black streaking (one isolate) in Bahman and Abadeh (Fras province).

As a whole, the highest frequency of *Phaeoacremonium* isolations was from brown internal necrosis (26%), followed by brown to black streaking (17.8%), watery necrosis (15.1%), dark brown wood discoloration just below the bark as well as wedge-shaped necrosis (13.7%) and arch-shaped necrosis (4.1%).

In the course of this study a number of additional fungi which had likely no bearing on induction of wood necrosis were occasionally isolated, i.e. *Paecilomyces variotii*, *Nattrassia mangiferae* (Syd. et P. Syd) B. Sutton et Dyko, *Cladosporium* sp., *Phoma* sp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Paecilomyces* sp., *Acremonium* sp., *Cytospora* spp., *Trichoderma* spp., *Alternaria* spp. and *Phaeoacremonium* sp.

Pathogenicity tests. All fungal isolates were pathogenic on detached shoots of grapevine ($F=48.84$, $P<0.0001$), apple ($F=146.11$, $P<0.0001$), pear ($F=97.02$, $P<0.0001$) and quince ($F=97.02$, $P<0.0001$ (not shown)). The average length of the vascular discoloration caused by *Phaeoacremonium* isolates in grapevine, apple, pear and quince are shown in Table 3. Although all fungal species caused wood discoloration, the aggressiveness varied among species. Isolates of *Pm. iranianum*, *Pm. parasiticum* and *Pm. aleophilum* were significantly more aggressive than the remaining fungal species on inoculated shoots of all species.

In grapevine, all isolates produced lesion lengths significantly longer than the negative control (2.6 mm). All *Pm. aleophilum* isolates were significantly more aggressive than the others and *Pm. iranianum*, *Pm. parasiticum*, *Pm. rubrigenum*, *Pm. scolyti* and *Pm. mortoniae* isolates followed in virulence in this order**.** *Pm scolyti* and *Pm. mortoniae* produced smaller lesions than the other species but differed significantly from controls. Reisolation from the inoculated shoots was successful with frequencies of 33.33% (*Pm. scolyti*, isolate PSPE1) to 100.0% (*Pm. parasiticum*, isolate PRPE1 and *Pm. iranianum*, isolate PIAP1)**.**

Pm. iranianum and *Pm. aleophilum* were the most pathogenic species on apple. *Pm. iraniaum* (isolate PIH1) in particular was the most aggressive with a mean lesion length of 34.0 mm. Other *Phaeoacremonium* species (with the exception of *Pm. mortoniae*) could also be considered pathogenic to apple, since their lesion lengths were significantly longer than the negative controls (3.3 mm). There was no statistically significant differences observed in the lesions produced by *Pm. scolyti* and *Pm. rubrigenum*. Of six *Phaeoacremonium* species only *Pm. mortinae* did not form significant lesions on apple (mean of 5.0 mm) as compared with the negative controls. Successful re-isolations ranged between 33.33% (*Pm. rubrigenum*, isolate PRUQ1) and 100.0% (*Pm. iranianum*, isolates PIAP1 and PIH1).

Pm. iranianum was the most agressive species on quince followed by *Pm. parasiticum*, *Pm. aleophilum*, *Pm. scolyti*, *Pm. rubrigenum* and *Pm. mortoniae*. *Pm. parasiticum*, *Pm. iranianum* and *Pm. aleophilum* had a high re-isolation rate, while *Pm. mortoniae* and *Pm. scolyti* were less frequently re-isolated.

Pm. iranianum was the most aggressive species on pear, followed by *Pm. parasiticum*, *Pm. aleophilum*, *Pm. scolyti*, and *Pm. rubrigenum*. *Pm. mortoniae* was not pathogenic to this host. No significant statistical difference was found between *Pm. parasiticum* (isolates PRAP1 and PRQ1) and *Pm. aleophilum*. Most species were re-isolated at frequencies between 91.67% (*Pm. iranianum*, isolate PIAP1) and 33.33% (*Pm. scolyti*, isolate PSQ1).

DISCUSSION

Trunk diseases of pome fruit trees and the associated pathogens have been little studied in Iran, thus the present study constitutes the first attempt to determine the presence in this country of *Phaeoacremonium* species in pome fruit trees showing various wood necrosis symptoms. Six *Phaeoacremonium* species were found, namely *Pm. aleophilum*, *Pm. parasiticum*, *Pm. iranianum*, *Pm. mortoniae*, *Pm. scolyti* and *Pm. rubrigenum*. *Pm aleophilum* was the most frequently isolated species, with an incidence of 42.5%. Interestingly, *Pm. aleophilum* is also the most common *Phaeoacremonium* species associated with Petri disease in grapevines (Mostert *et al*., 2006a; Essakhi *et al*., 2008; Gramaje *et al*., 2009), and the prevailing species associated with vine decline in Iran (Mohammadi and Banihashemi, 2007; Mohammadi *et al*., 2013). Five of the six *Phaeoacremonium* species found during this study had already been recorded from grapevine in Iran (Mohammadi *et al*., 2013; Mohammadi and Banihashemi, 2012). with the exception of *Pm. scolyti.*

Pm. aleophilum, *Pm. iranianum*, *Pm. parasiticum* and *Pm. mortoniae* were found in apple trees, from which only *Pm. aleophilum*, *Pm. angustius* and *Pm. mortoniae* had previously been reported (Rooney-Latham *et al*., 2006; Cloete *et al*., 2011). It ensues that this is the first report of *Pm. iranianum* and *Pm. parasiticum* in apple.

As to pear, *Pm. aleophilum*, *Pm. rubrigenum*, *Pm. scolyti* and *Pm. parasiticum* were isolated from Iranian trees showing decline symptoms, whereas Cloete *et al.* (2011), had reported the occurrence of *Pm. aleophilum*, *Pm. iranianum*, *Pm. mortoniae* and *Pm. viticola* on the same host in South Africa. Thus, this is the first Iranian record of *Pm. rubrigenum*, *Pm. scolyti* and *Pm. parasiticum* in pear. Likewise, the isolation of *Pm. aleophilum*, *Pm. iranianum*, *Pm. rubrigenum*, *Pm. scolyti* and *Pm. parasiticum* from quince trees, represents the first record of all of these species on this host.

In Iran, *Pm. rubrigenum* had previously been found in declining persimmon (*Diospyros kaki*) trees in Shiraz (Fars province) (Jamali and Banihashemi, 2012), and more

Table 2. Geographical origin, associated external and internal symptoms and number of fungal isolates recovered from pome fruit trees in the Iranian provinces of Fars and Kerman.

Location	Internal lesion types[b]							External symptoms[a]	Pome fruit trees				Isolates	
	7	6	5	4	3	2	1		Hawthorn	Quince	Pear	Apple	Numbers (percentage)	Identity
Kerman, Baft, Shiraz, Abadeh, Eghlid, Najaf Abade, Sadegh Abad, Bahman, Soghad, Mohy Abad, Sirjan	3	3	3	4	5	8	5	Y,DI,D,W, C,DF	0	10	7	14	31 (16.1%)	*Phaeoacremonium aleophilum*
Kerman, Baft, Abadeh, Eghlid, Shiraz, Mohy Abad	1	7	2	0	3	4	2	W,D,DI,C,DF	0	11	2	6	19 (9.8%)	*Phaeoacremonium parasiticum*
Shiraz, Abadeh	0	0	0	5	0	3	0	DI,DF	3	4	0	1	8 (4.1%)	*Phaeoacremonium iranianum*
Abadeh, Baft	0	3	0	0	4	0	0	DI,DF	0	4	3	0	7 (3.6%)	*Phaeoacremonium rubrigenum*
Bahman, Sadegh Abad	0	0	0	0	1	4	0	DI	0	0	0	5	5 (2.6%)	*Phaeoacremonium mortoniae*
Abadeh	0	3	0	0	0	0	0	DF,DI	0	2	1	0	3 (1.6%)	*Phaeoacremonium scolyti*
Shiraz, Kerman	0	3	1	0	5	0	2	C, DI,DF	3	5	1	2	11 (5.7%)	*Paecilomyces variotii*
Abadeh, Shiraz, Mohy Abad	0	3	2	0	1	5	3	DI,D,Y	0	2	5	7	14 (7.3%)	*Nattrassia mangifera*e
Kerman, Abadeh	0	4	0	0	3	1	0	D,C,Y	0	1	0	7	8 (4.1%)	*Cladosporium* sp.
Shiraz, Kerman	0	4	0	0	0	0	1	DI,D	0	4	0	1	5 (2.6%)	*Phoma* sp.
Shiraz, Abadeh	0	3	0	1	2	0	5	C,DI	2	2	3	4	11 (5.7%)	*Fusarium* spp.
Dashte-Arjan	1	0	3	1	3	1	4	DF,D,C,Y	5	1	5	2	13 (6.7%)	*Aspergillus* sp.
Dashte-Arjan, Shiraz	3	0	2	0	3	0	2	C,Y,W,DI	3	1	3	3	10 (5.2%)	*Penicillium* sp.
Abadeh	0	0	0	0	0	0	2	DI	0	0	0	2	2 (1.1%)	*Phaeoacremonium* sp.
Shiraz, Bahman	0	5	0	0	0	1	1	C,DI,W	0	0	5	2	7 (3.6%)	*Acremonium* sp.
Soghad, Bahman, Shiraz	0	0	4	0	0	3	0	DI,D,Y	0	5	0	2	7 (3.6%)	*Cytospora* spp.
Shiraz, Abadeh, Kerman, Sirjan, Baft	0	4	0	4	0	1	2	C,DI,D	1	0	2	8	11 (5.7%)	*Trichoderma* spp.
Abadeh, Eghlid, Dashte-Arjan	0	5	1	2	0	4	0	C,Y,W,DI	2	1	5	4	12 (6.2%)	*Alternaria* spp.
Shiraz, Baft, Abadeh, Dashte-Arjan, Eghlid	0	2	4	0	3	0	0	DF,D,C,Y	0	7	1	1	9 (4.7%)	*Paecilomyces* sp.

[a] Summary of observed external symptoms in the original materials: C= canker, D= decline, DF=defoliation, DI= dieback, W=wilting and Y= yellowing.

[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 just below the bark, 6= wedge-shaped necrosis., 7= arch shaped necrosis.

Table 3. Mean lesion length and re-isolation frequencies of *Phaeoacremonium* species inoculated onto detached shoots of grapevine, apple, quince and pear in pathogenicity tests.

Phaeoacremonium species	Isolates inoculated			Original host	Mean lesion length (mm) [b]				Re-isolation frequency %			
	KER-U no.[a]	Code	Accession numbers		Grapevine	Apple	Quince	Pear	Grapevine	Apple	Quince	Pear
Pm. parasiticum	PRAP1	PHAPL2	KF467603	Apple	16.1 **cd**	19.6 **c**	21.6 **cd**	27.8 **cb**	91.67	91.67	100	75.00
Pm. parasiticum	PRQ1	PHQU3	KF467604	Quince	15.6 **d**	14.5 **d**	21.2**cde**	26.1 **c**	83.33	91.67	100	75.00
Pm. parasiticum	PRPE1	PHPEA4	KF467602	Pear	15.1 **de**	21.0 **c**	20.4 **cde**	28.8 **ab**	100	66.67	100	75.00
Pm. aleophilum	PALAP1	PHAPL5	KF467612	Apple	24.0 **a**	31.9 **ab**	30.9 **b**	25.8 **c**	91.67	66.67	83.33	83.33
Pm. aleophilum	PALPE1	PHPEA3	KF467610	Pear	23.6 **a**	30.9 **ab**	30.0 **b**	26.0 **c**	91.67	75.00	91.67	75.00
Pm. aleophilum	PALQ1	PHQU2	KF467611	Quince	23.5 **a**	30.6 **b**	23.8 **c**	26.5 **c**	66.67	91.67	83.33	83.33
Pm. iranianum	PIAP1	PHIAPL4	KF467608	Apple	20.4 **b**	33.6 **ab**	36.6 **a**	30.9 **ab**	100	100	83.33	91.67
Pm. iranianum	PIQ1	PHIQU2	KF467609	Quince	19.9 **b**	33.0 **ab**	37.0 **a**	31.0 **a**	83.33	91.67	100	83.33
Pm. iranianum	PIH1	PHIOW1	KF467607	Hawthorn	19.1 **bc**	34.0 **a**	36.0 **a**	30.8 **ab**	91.67	100	100	75.00
Pm. rubrigenum	PRUPE1	PHUPEA2	KF467600	Pear	13.9 **edf**	15.8 **d**	15.0 **f**	13.8 **d**	50.00	83.33	75.00	58.33
Pm. rubrigenum	PRUQ1	PHUPEA1	KF467601	Quince	13.3 **edf**	13.1 **d**	15.7 **f**	13.1 **d**	58.33	33.33	75.00	66.67
Pm. mortoniae	PMOAP1	PHMAPL2	KF467606	Apple	11.3 **f**	5.0 **e**	14.3 **f**	4.6 **e**	66.67	41.67	33.33	75.00
Pm. scolyti	PSPE1	PHSPEA1	KF467598	Pear	11.5 **f**	15.4 **d**	17.1 **ef**	14.5**d**	33.33	75.00	58.33	66.67
Pm. scolyti	PSQ1	PHSQU2	KF467599	Quince	12.3 **ef**	15.2 **d**	17.7 **def**	15.1 **d**	58.33	41.67	75.00	33.33
PDA plug					2.6 **g**	3.3 **e**	3.40 **g**	3.6 **e**	–	–	–	–
LSD (P<0.01)					3.0786	3.2283	4.1636	3.1552				

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

[b] Lesions lengths followed by the same letter were not significantly different.

recently in grapevine and cypress (*Cupressus sempervirens* L.) (Mohammadi, 2013; Mohammadi *et al*., 2014). On the other hand, *Pm. iranianum* was the only *Phaeoacremonium* species isolated from hawthorn. Therefore, quince and hawthorn are new woody hosts for *Phaeoacremonium* in the world.

Pm. viticola was not isolated from pome fruit trees, but this species has previously been reported from grapevine in Iran (Mostert *et al*., 2006a).

In the present study, seven different types of wood necrosis were observed in cross-sectioned branches of pome fruit trees, which were referred to as black spots, wedge-shaped necrosis, brown internal necrosis, watery necrosis, brown to black streaking, dark brown wood discoloration just below the bark and arch-shaped necrosis. Similar symptoms were also reported for grapevine trunk diseases (Van Niekerk *et al*., 2011). The present survey has also shown the frequent occurrence of internal symptoms associated with a declining condition pome fruit trees, as reported for grapevine trunk diseases (Mugnai *et al*., 1999). All symptom types (with the exception arch-shaped necrosis) have previously been observed in Iranian grapevines (Mohammadi and Banihahsemi, 2007, 2012; Mohammadi, 2012; Mohammadi *et al*., 2013). Cloete *et al.* (2011) identified six different types of symptoms, i.e brown vascular streaking, black vascular streaking, wedge-shaped necrosis, watery necrosis, brown internal necrosis and soft rot associated with apple and pear trunk diseases in South Africa. In general, *Phaeoacremoniu*m species can be isolated from different kinds of lesions associated with trunk diseases. In the present case, *Phaeoacremonium* species were mostly recovered from brown internal necrosis, which is consistent with previous studies (Larignon and Dubos, 1997; Luque *et al*., 2009). In particular, *Pm. aleophilum* was isolated from all internal symptoms types while *Pm. scolyti* was only obtained from wedge-shaped necrosis. On the contrary, Berraf-Tebbal *et al.* (2011) isolated *Phaeoacremonium* species more frequently from V-shaped necrosis of grapevine in Algeria.

Pathogenicity tests revealed a variation in lesion lengths and re-isolation frequencies between fungal species and hosts. According to pathogenicity tests, most *Phaeoacremonium* species were shown to be potentially pathogenic. On grapevine shoots, *Pm. aleophilum* caused the longest lesions while *Pm. mortoniae* caused the smallest. *Pm. aleophilum* is considered as one of the main grapevine pathogens involved in the "esca" and Petri disease (Mostert *et al*., 2006a). This species together with *Pm. parasiticum*, *Pm. viticola* and *Pm. subulatum* were shown to be true pathogens and colonizers of grapevine wood (Sparapano *et al*., 2001; Halleen *et al*., 2007).

The lesions caused by *Pm. iranianum* on apple, pear and quince were significantly longer than those caused by other species. Cloete *et al.* (2011) obtained similar results when testing the pathogenicity of *Pm. iranianum* on detached apple shoots. *Pm. mortoniae* was not pathogenic to pear and apple, but can be considered as pathogenic to grapevine and quince. Aroca and Raposo (2009) also found that this species can cause severe vascular discoloration on cuttings of *V. vinifera* cv. Monastrell. On the contrary, Cloete *et al.* (2011) found *Pm. mortoniae* to be pathogenic to apple as it caused lesions significantly longer than the negative controls. Several reasons can explain these differences in virulence, including isolate variability and inoculation conditions used in pathogenicity tests.

Species of *Phaeoacremonium* are well known as endophytes and trunk pathogens of numerous woody hosts. Although most species of *Phaeoacremonium* are associated with necrosis and discolorations of grapevine wood, recent studies have shown the occurrence of several *Phaeoacremonium* species on other woody hosts such as olive (Hawksworth *et al*., 1976), kiwifruit (Di Marco *et al*., 2004), pome and stone fruit trees (Damm *et al*., 2008; Cloete *et al*., 2011) and almond trees (Gramaje *et al*., 2012). Thus, the association of *Pm. aleophilum* and *Pm. parasiticum* with three different pome fruit trees, apple, pear and quince in Iran is not surprising.

The broad host range of *Phaeoacremonium* spp. and lack of host specialization is likely to hinder trunk disease management strategies. To our knowledge, this article is the first report of *Pm. scolyti* from Iran. This species has previously been reported from grapevine (Mostert *et al*., 2006a). According to our pathogenicity tests, this species was pathogenic to grapevine, therefore pome fruit trees can act as alternative hosts providing a reservoir from which grapevine infection can occur**.** Based on the results obtained in this study on pome fruit trees, a number of *Phaeoacremonium* spp., which have previously been reported to be pathogenic to grapevines were isolated and identified from the symptomatic wood of apple, quince, pear and hawthorn in Iran. Therefore pome fruit orchards should be considered as potential inoculum sources of trunk disease pathogens to grapevines planted in close proximity to these woody trees.

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