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Trunk Disease Fungi Associated with *Syzygium cumini* in Iran

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Abstract

Syzygium cumini trees with dieback symptoms and cankers were observed in two provinces in Iran. Isolations were made from diseased branches and cankers and from asymptomatic *S. cumini* wood samples. Several trunk disease pathogens were identified based on morphological characteristics and by molecular methods, including *Cadophora luteo-olivacea*, *Diplodia sapinea*, *D. seriata*, *Neoscytalidium hyalinum*, *Phaeoacremonium fraxinopennsylvanicum*, *P. krajdenii*, *P. parasiticum, P. viticola*, and *Pleurostoma richardsiae*, which were isolated from *S. cumini* for the first time in the world. Pathogenicity tests conducted with all species confirmed their status as possible *S. cumini* pathogens. *N. hyalinum* was the most aggressive species and caused the longest lesions on inoculated shoots. The endophytic character of some fungal species isolated from asymptomatic wood of *S. cumini* is further discussed. Our results indicated that *S. cumini* is a new woody host to many known fungal trunk pathogens.

The genus *Syzygium* (family Myrtaceae) contains about 1,200 to 1,500 species (Craven and Biffin 2010). Several genera of Myrtaceae, including *Syzygium*, are well known for their economic importance and are cultivated worldwide for their fleshy fruit. *Syzygium cumini* (L.) Skeels (synonym: *Eugenia jambolana*) is one of the most common medicinal plants in various traditional systems of medicine. Fruits of this species are highly nutritive and contain various useful components such as carbohydrates, vitamins, antioxidant compounds, proteins, fats, amino acids, and minerals that are essential for human health and play important roles in different functions of the human body (Modi et al. 2010; Prabhakaran et al. 2011). In Iran, *S. cumini* is used as an ornamental and shade tree and is restricted to southern areas, where its fruits are also used by indigenous people.

The risk posed by the emergence of a broad range of taxonomically unrelated trunk disease pathogens to the sustainability of fruit and tree nut industries worldwide has been reported by Gramaje et al. (2016). Recent studies demonstrated that forest and ornamental systems are also susceptible to fungal trunk pathogen attack (Hashemi et al. 2017; Jankowiak et al. 2016; Kazemzadeh Chakusary et al. 2017; Mora-Sala et al. 2018). For instance, species of *Eucalyptus*, the most widely studied genus in the family Myrtaceae, represent a rich catch-crop for many fungal trunk pathogens, especially Botryosphaeriaceae spp. (Pérez et al. 2010; Slippers et al. 2004). Few studies are available about the incidence of fungal trunk pathogens in *Syzygium*, a closely related genus to *Eucalyptus*. A number of Botryosphaeriaceae spp. have been reported as pathogens of *Syzygium cordatum* in South Africa (Pavlic et al. 2004, 2007) and *Syzygium paniculatum* in Florida (Ploetz et al. 2009). The canker pathogens *Chrysoporthe austroafricana* and *C. cubensis* have been isolated from several *Syzygium* spp. in South Africa (Heath et al. 2006) and South China (Chen et al. 2010), respectively, including *S. cumini* (Chen et al. 2010).

Over the last 10 years, several species of the Botryosphaeriaceae family and the genus *Phaeoacremonium* have been reported from various woody trees in Iran (Hashemi and Mohammadi 2016; Hashemi et al. 2017; Kazemzadeh Chakusary et al. 2017; Mohammadi et al. 2013). However, there is no information about the incidence of fungal trunk pathogens on *S. cumini* trees. In 2014, severe decline of *S. cumini* trees was noticed in several provinces in Iran. Disease symptoms included yellowing, shoot dieback, and branch and trunk cankers. Internal wood symptoms ranged from wedge-shaped necroses to brown vascular streaking visible as circular discoloration of the xylem vessels. Therefore, the aim of this study was to determine the etiology of fungal trunk pathogens associated with wood necroses of *S. cumini* in Iran and to evaluate their status as pathogens on this host by conducting pathogenicity tests under greenhouse conditions.

Materials and Methods

Sampling and collection of fungal isolates. Between 2014 and 2016, several surveys were conducted throughout Kerman (29.4850° N, 57.6439° E) and Hormozgan (27.1387° N, 55.1376° E) provinces with the aim of isolating and identifying fungal trunk pathogens on *S. cumini*. Forty-seven branches showing yellowing, cankers, and dieback and 21 asymptomatic branches were collected from 38 trees at various sites in Kahnouj (28.0250° N, 57.7460° E) and Boluk (28.2314° N, 57.5151° E) in Kerman province, and Rudan (27.4381° N, 57.1803° E) in Hormozgan province. One to three samples from each tree were collected. Fungal isolations were made from asymptomatic twigs as well as from the edges of necrotic wood lesions of trees displaying disease symptoms. From each sample, 10 to 15 small fragments (approximately 5 to 7 mm) were cut and surface disinfected by immersing in 1.5% solution of NaOCl for 30 s and rinsed in sterile distilled water. Woody tissues were taken and placed into Petri dishes containing potato dextrose agar (PDA, 39 g/liter, Merck) supplemented with 100 mg/liter of streptomycin sulfate. Plates were incubated at 25°C in the dark until mycelial growth could be detected from wood pieces. All cultures were obtained either by single-spore or hyphal-tip methods prior to morphological and molecular identification of the isolates. Pure cultures were maintained in the culture collection of the Plant Protection Department at Shahid Bahonar University of Kerman, Kerman, Iran.

Morphological identification of fungal isolates. All fungal isolates were initially characterized and classified on the basis of their microscopic structures and cultural appearance on PDA. Culture characters and pigment production on PDA, malt extract agar (MEA; Merck), and oatmeal agar (30 g of oatmeal, 15 g of agar; Merck) and main microscopic structures (phialide type and shape, conidiophore morphology, hyphal wart size, and conidial shape and size) from aerial mycelium were used to identify *Phaeoacremonium* isolates (Marin-Felix et al. 2018). The main morphological

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characters such as conidiophore, phialide, collarettes, and conidial morphology were used to identify *Pleurostoma* (Réblová et al. 2015; Vijaykrishna et al. 2004) and *Cadophora* (Harrington and McNew 2003) isolates. Species of Botryosphaeriaceae were identified based on colony morphology, colony color and growth, conidial shape and color, the presence or absence of septa, and cell wall structure (Phillips et al. 2013). To enhance sporulation, five to seven mycelial plugs (4-mm diameter) of each Botryosphaeriaceae isolate were placed in 80-mm Petri dishes containing 2% water agar (2% agar; Merck) supplemented with sterilized pine needles. Plates of each isolate were incubated under continuous light at 25°C until anamorph structures (pycnidia) were produced on the pine needles. All the main microscopic structures were mounted on glass microscope slides and studied in more detail under a standard light microscope (BH2, Olympus Optical, Tokyo, Japan).

DNA isolation, amplification, and sequencing of fungal isolates. Representative isolates from each fungal group were selected for molecular studies. Pure cultures of each selected isolate were subcultured on PDA and incubated at 25°C for 8 to 16 days. For each isolate, 45 to 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 an AccuPrep_Genomic DNA Extraction Kit (Bioneer, Daejeon, South Korea) following the manufacturer's instructions. Obtained DNA was separated on 0.1% agarose gels stained with ethidium bromide and visualized under ultraviolet light. All DNA tubes were stored at –20°C until further use. The internal transcribed spacers (ITS1 and ITS2) including the 5.8S ribosomal RNA gene were amplified to confirm identity of Botryosphaeriaceae, *Pleurostoma*, and *Cadophora* isolates. For the Botryosphaeriaceae spp., a partial sequence of translation elongation factor 1-α (TEF-1a) was also amplified and sequenced. The ITS region and TEF-1a gene were amplified using primers pairs ITS1/ITS4 (White et al. 1990) and EF1-728F/EF1-986R (Carbone and Kohn 1999), respectively. The *Phaeoacremonium* spp. were identified by sequence analysis of the β-tubulin (BT) and actin (ACT) genes, using the primer pairs T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995), and ACT-512F and ACT-783R (Carbone and Kohn 1999). Polymerase chain reaction (PCR) mixtures for amplification of selected loci of the isolates consisted of 1× PCR buffer, 200 μM of each dNTP, 0.5 μM of each primer, 1.5 mM $MgCl_2$, 1.25 U of DNA Taq polymerase (Cinnagen, Tehran, Iran), and 1 μl of each template DNA. Sterile water (Chromasolv Plus [Sigma-Aldrich, Steinheim, Germany]) was added to adjust the mixes to a final volume of 25 μl. All amplification reactions were conducted on a Techne TC-312 Thermal Cycler (Techne, Cambridge, U.K.) as described by Hashemi and Mohammadi (2016). The PCR products were visualized on 1% agarose gels (UltraPure Agarose; Invitrogen, Carlsbad, CA) in TAE buffer. A 100-bp ladder (GeneRuler DNA Ladder Mix, Fermentas, Vilnius, Lithuania) was used as a molecular weight marker to estimate the size of PCR products. PCR products were purified and sequenced in both directions by Bioneer Corporation (Daejeon, South Korea). Fungal species were initially identified using the MegaBLAST function of the National Center for Biotechnology Information's GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/).

Phylogenetic analysis. A phylogenetic analysis was performed only for *Phaeoacremonium* isolates. Sequences used by Spies et al. (2018) were included as reference sequences. *Calosphaeria africana* CBS 120870, *Jattaea algeriensis* CBS 120871, and *Pleurostoma richardsiae* CBS 270.33 were included as outgroups. Reference and de novo-generated sequences of the two gene regions were aligned separately using MAFFT sequence alignment program version 6 (Katoh and Toh 2010), followed by manual adjustments of the alignments in Sequence Alignment Editor version 2.0a11. A partition homogeneity test was conducted in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2003). The congruence between the ACT and BT datasets were tested at 1,000 replicates. Maximum likelihood was performed on the concatenated alignment.

Maximum likelihood analysis was performed in MEGA version 7 software (Kumar et al. 2016) under the best fit model (HKY+I+G) as estimated with the Bayesian information criterion in jModeltest2 (Darriba et al. 2012). Branch support was calculated from 100 bootstrap replicates for the concatenated dataset only. *Phaeoacremonium* sequences derived in this study were lodged at GenBank (Table 1) and the alignments in TreeBASE (https://www.treebase.org).

Pathogenicity tests. Twelve fungal isolates consisting of *Neoscytalidium hyalinum*, *Diplodia seriata*, and *Phaeoacremonium fraxinopennsylvanicum* (two isolates from each species), *D. sapinea*, *P. parasiticum*, *P. krajdenii*, *P. viticola*, *Pl. richardsiae*, and *Cadophora luteo-olivacea* (one isolate from each species) obtained from diseased and healthy trees in this study were selected for pathogenicity tests on *S. cumini*. In total, 104 detached woody shoots (ranging from 35 to 40 cm in height and 2 to 2.5 cm in diameter) were collected from 15 symptomless trees and were used for pathogenicity trials under controlled conditions. Selected shoots were surface disinfected with 70% ethanol, and a superficial hole with a 4-mm-diameter cork borer was then made at the inoculation point. A mycelial plug (4 mm in diameter and 3 mm thick) taken from the margin of a growing colony on PDA (7 to 20 days old) was put into each hole. Each inoculation point was protected by moist cotton and wrapped with a strip of Parafilm (Pechiney Plastic Packaging, Menasha, WI) to prevent desiccation. Experiments were laid down following a completely randomized design with eight replications for each fungal isolate and repeated once under similar conditions. Eight shoots were wounded and inoculated in a similar manner with sterile PDA plugs, which served as controls. The bases of inoculated shoots were inserted into containers filled with water (1,000 to 1,500 ml), covered with a transparent plastic bag, and maintained at room temperature (25 ± 2°C). Lengths of wood lesions induced by inoculated isolates were evaluated 45 days after inoculation. All data of the pathogenicity tests were subjected to analysis of variance using SAS version 9.1 (SAS Institute, Cary, NC). Fungal reisolations were carried out from the edges of produced lesions on PDA as described previously.

Results

Sampling and collection of fungal isolates. Both external and internal disease symptoms were observed on the surveyed trees and included yellowing, cankers on branches and trunks (Fig. 1A and B), and branch dieback (Fig. 1A). In cross sections, the most common symptoms were black spots (Fig. 1C, b), watery necrosis (Fig. 1D, c), wedge-shaped necrosis (Fig. 1E, a), arch-shaped necrosis (Fig. 1E, c), central necrosis (Fig. 1E, d), brown to black wood streaking (Fig. 1F, a), irregular wood necrosis (Fig. 1F, b), and wood decay (Fig. 1F, c). No internal wood symptoms were observed in cross sections of apparently healthy branches.

Overall, 72 fungal isolates were recovered from *S. cumini*. Four species of Togniniaceae family (*P. parasiticum*, *P. krajdenii*, *P. viticola*, and *P. fraxinopennsylvanicum*), three species of Botryosphaeriaceae family (*Diplodia seriata*, *D. sapinea*, and *Neoscytalidium hyalinum*), one species of Pleurostomataceae family (*Pl. richardsiae*), and *Cadophora luteo-olivacea* were isolated from symptomatic and asymptomatic branches of *S. cumini.* Fungal species were recovered from trees at frequencies between 2.8% (*D. sapinea*) to 23.6% (*N. hyalinum*) (Table 2). Eight wood lesion types were recorded on wood samples collected from *S. cumini*. Most of the fungal isolates were recovered from irregular wood necrosis (26.3%) and central necrosis (24.6%). No fungal isolate was obtained from wood decay. Of the five *Phaeoacremonium* species, *P. fraxinopennsylvanicum* was isolated from seven wood lesion types, whereas *P. viticola* was recovered only from two types of wood lesions (irregular wood necrosis and central necrosis). Of the three species of Botryosphaeriaceae, *N. hyalinum* was obtained from six wood lesion types, and *D. seriata* was isolated only from irregular wood necrosis and wedge-shaped necrosis. Both species of *Pl. richardsiae* and *C. luteo-olivacea* were only associated with irregular wood necrosis and central necrosis. Fifty-seven fungal isolates (79.2% of total isolates) were recovered from discolored tissues of *S. cumini*. Of these, 28.9% represented *N. hyalinum*, 27.8% *P. fraxinopennsylvanicum*,

12.3% *P. krajdenii*, 8.8% *P. parasiticum*, 7.0% *Pl. richardsiae*, and 5.3% for each species of *P. viticola*, *D. seriata*, and *C. luteo-olivacea* (Table 2). Regarding wood lesion types, the most frequent *Phaeoacremonium* isolates (10/15 isolates: 66.7%) were obtained from central necrosis, whereas most isolates of Botryosphaeriaceae were associated with wedge-shaped necrosis (8/20 isolates: 40.0%). The highest isolation frequencies of *Pl. richardsiae* (3/4 isolates: 75.0%) and *C. luteo-olivacea* (2/3 isolates: 66.7%) isolates were recorded from irregular wood necrosis.

Fifteen fungal isolates (20.8%) were obtained from asymptomatic branches of trees. *N. hyalinum* was the most frequently obtained species (33.3%) from healthy tissues, followed by *D. seriata* and *P. fraxinopennsylvanicum* (26.7%) and *D. sapinea* (13.3%). *P. parasiticum*, *P. viticola*, *P. krajdenii, Pl. richardsiae*, and *C. luteo-olivacea* were recovered from discolored wood tissues. *P. fraxinopennsylvanicum*, *N. hyalinum*, and *D. seriata* were isolated from both healthy and discolored wood tissue types. In this study, we found *D. sapinea* only in healthy tissues.

Morphological identification and characterization of fungal isolates. Based on morphological criteria (culture and microscopic characterization) the main fungal isolates obtained from *S. cumini* were classified into *Phaeoacremonium*, *Diplodia*, *Neoscytalidium*, *Pleurostoma*, and *Cadophora* genera. Thirty-five isolates were characterized by pale brown to reddish brown, flat, slow-growing cultures on MEA, abundant sporulation, and hyaline and aseptate conidia. Septate hyphae were single or fasciculate. Three types of phialides, variable in size and shape, were observed in these isolates. All morphological characters corresponded to the genus *Phaeoacremonium* (Marin-Felix et al. 2018; Mostert et al. 2006).

Eighteen isolates formed white, dark green, or gray to dark gray fast-growing colonies on PDA. These isolates also produced black and globose fruiting bodies (pycnidia) on pine needles after 15 to 30 days. Conidia were pigmented or hyaline. These morphological and cultural characteristics corresponded to the family Botryosphaeriaceae (Phillips et al. 2013). At the genus level, these isolates were assigned to two genera: *Diplodia* and *Neoscytalidium*.

Five isolates of a fungus resembling the *Phialophora*-like anamorph were obtained in this study. These isolates formed brown to olive-brown colonies on MEA. Conidiophores were single, hyaline to pigmented, and phialides were long with prominently flaring collarettes. Two types of conidia, brown, (sub)globose and hyaline, allantoid to cylindrical conidia were produced by this group of isolates. These characters were consistent with the description of *Pl. richardsiae* (Réblová et al. 2015; Vijaykrishna et al. 2004). Eight isolates of a phialidic fungus were isolated from the symptomatic plants. Colonies on PDA were flat, felty, and varying in color from white to gray and black-olivaceous. Conidia were ellipsoid or elongate.Cultural and morphological characteristics of these isolates were similar to those described for *Cadophora* spp. (Gramaje et al. 2011; Harrington and McNew 2003).

Molecular characterization and phylogenetic analyses. According to ITS sequences and BLASTn searches in GenBank, *Phialophora*-like anamorph isolates had 99 to 100% identity with isolates of *Pl. richardsiae*. ITS and TEF sequences of Botryosphaeriaceae isolates were similar to isolates previously identified as *N. hyalinum, D. seriata*, and *D. sapinea* in GenBank. ITS sequences of *Cadophora* isolates were identical to isolates previously described as *C. luteo-olivacea* in GenBank. Percentage homology of some representative isolates, GenBank accession numbers, and related

Table 1. Origin and GenBank accession numbers of *Phaeoacremonium* isolates obtained from *Syzygium cumini* (used in phylogenetic studies)

Isolates				GenBank accession number	
Species	**Culture code**	**Collector**	**Locality**	**β-Tubulin**	**Actin**
P. krajdenii	IRNHM-KSP1	S. Panahandeh	Kahnouj, Kerman province	MH252089	MH236351
	IRNHM-KSP2	S. Panahandeh	Kahnouj, Kerman province	MH252090	MH236352
	IRNHM-KSP3	S. Panahandeh	Kahnouj, Kerman province	MH252091	MH236353
	IRNHM-KSP4	S. Panahandeh	Kahnouj, Kerman province	MH252092	MH236354
	IRNHM-KSP5	S. Panahandeh	Kahnouj, Kerman province	MH252093	MH236355
	IRNHM-KSP6	S. Panahandeh	Kahnouj, Kerman province	MH252094	MH236356
	IRNHM-KSP7	S. Panahandeh	Kahnouj, Kerman province	MH252095	MH236357
P. parasiticum	IRNHM-KSP8	S. Panahandeh	Kahnouj, Kerman province	MH252096	MH236358
	IRNHM-KSP9	S. Panahandeh	Kahnouj, Kerman province	MH252097	MH236359
	IRNHM-KSP10	S. Panahandeh	Boluk, Kerman province	MH252098	MH236360
	IRNHM-KSP11	S. Panahandeh	Kahnouj, Kerman province	MH252099	MH236361
	IRNHM-KSP12	S. Panahandeh	Boluk, Kerman province	MH252100	MH236362
P. viticola	IRNHM-KSP13	H. Mohammadi	Rudan, Hormozgan province	MH252101	MH236363
	IRNHM-KSP14	H. Mohammadi	Rudan, Hormozgan province	MH252102	MH236364
	IRNHM-KSP15	H. Mohammadi	Rudan, Hormozgan province	MH252103	MH236365
P. fraxinopennsylvanicum	IRNHM-KSP16	S. Panahandeh	Kahnouj, Kerman province	MH252104	MH236366
	IRNHM-KSP17	H. Mohammadi	Rudan, Hormozgan province	MH252105	MH236367
	IRNHM-KSP18	S. Panahandeh	Boluk, Kerman province	MH252106	MH236368
	IRNHM-KSP19[z]	H. Mohammadi	Rudan, Hormozgan province	MH252107	MH236369
	IRNHM-KSP20	S. Panahandeh	Boluk, Kerman province	MH252108	MH236370
	IRNHM-KSP21	S. Panahandeh	Kahnouj, Kerman province	MH252109	MH236371
	IRNHM-KSP22	H. Mohammadi	Rudan, Hormozgan province	MH252110	MH236372
	IRNHM-KSP23[z]	H. Mohammadi	Rudan, Hormozgan province	MH252111	MH236373
	IRNHM-KSP24	S. Panahandeh	Boluk, Kerman province	MH252112	MH236374
	IRNHM-KSP25	H. Mohammadi	Rudan, Hormozgan province	MH252113	MH236375
	IRNHM-KSP26	H. Mohammadi	Rudan, Hormozgan province	MH252114	MH236376
	IRNHM-KSP27	H. Mohammadi	Rudan, Hormozgan province	MH252115	MH236377
	IRNHM-KSP28	H. Mohammadi	Rudan, Hormozgan province	MH252116	MH236378
	IRNHM-KSP29	H. Mohammadi	Rudan, Hormozgan province	MH252117	MH236379
	IRNHM-KSP30[z]	H. Mohammadi	Rudan, Hormozgan province	MH252118	MH236380
	IRNHM-KSP31	S. Panahandeh	Kahnouj, Kerman province	MH252119	MH236381
	IRNHM-KSP32	S. Panahandeh	Kahnouj, Kerman province	MH252120	MH236382
	IRNHM-KSP33[z]	H. Mohammadi	Rudan, Hormozgan province	MH252121	MH236383
	IRNHM-KSP34	H. Mohammadi	Rudan, Hormozgan province	MH252122	MH236384

[z] Isolates obtained from asymptomatic wood tissues of *S. cumini*.

references in GenBank are given in Table 3. For *Phaeoacremonium* isolates, maximum likelihood of the combined ACT-BT regions yielded a phylogeny with high support (98 to 100% maximum likelihood bootstrap) for all species-level clades except *P. alvesii* (paraphyletic, 82% bootstrap support), *P. griseorubrum* (paraphyletic, 64% bootstrap support), *P. roseum* (87% bootstrap support), and *P. viticola* (paraphyletic with regard to *P. angustius* and *P. roseum*). The 34 Iranian strains clustered in four clades (*P. parasiticum*, *P. krajdenii*, *P. fraxinopennsylvanicum*, and *P. viticola* clades) with 99% bootstrap support (Fig. 2).

Pathogenicity tests. Mean lesion lengths caused by the inoculated isolates compared with the control treatment are shown in Table 4. Forty-five days after inoculation, brown to black wood lesions showing typical wood discoloration were observed upward and downward from inoculation points in detached shoots of *S. cumini*. Based on the results, lesion lengths produced by the inoculated isolates varied among the fungal species. Two isolates of *N. hyalinum*, namely, IRNHM-KSP35 (53.75 mm) and IRNHM-KSP40 (49.13 mm) were the most aggressive isolates and produced the largest lesions on inoculated shoots. In contrast, two isolates of *D. seriata,* namely, IRNHM-KSP60 (7.25 mm) and IRNHM-KSP59 (6.50 mm) produced the smallest wood lesions. No significant difference was found between IRNHM-KSP59 isolate and the control (4.63 mm) ($P > 0.05$). Four isolates, namely, IRNHM-KSP40 (*N. hyalinum*), IRNHM-KSP57 (*D. sapinea*), IRNHM-KSP60 (*D. seriata*), and IRNHM-KSP19 (*P. fraxinopennsylvanicum*) were collected from healthy tissues, even though they were shown to be pathogenic on inoculated shoots. All inoculated species caused longer basipetal than acropetal lesions in all treatments (Fig. 3). Ten isolates of eight species produced upward and downward wood lesions that were significantly different in size to those in the control ($P < 0.05$). Two isolates of *D. seriata* (one obtained from healthy tissues and another from necrotic wood tissues) did not produce any significant lesion lengths both in upward and in downward directions on the inoculated shoots. Fungal reisolation frequencies of the fungal species inoculated onto detached woody shoots of *S. cumini* ranged from 38.9% (*Pl. richardsiae*) to 94.4% (*N. hyalinum*) (Table 4). None of the fungal species were recovered from the control shoots.

Fig. 1. External and internal wood disease symptoms on *Syzygium cumini*: **1,** branch dieback (arrow); **2,** trunk canker (white dashed lines); **3,** central necrosis (a) and black spots (b); **4,** irregular wood necrosis (a), black wood streaking (b), and watery necrosis (c); **5,** wedge-shaped necrosis (a), watery necrosis (b), arch-shaped necrosis (c), and central necrosis (d); and **6,** black wood streaking (a), irregular wood necrosis (b), and wood decay (c).

Discussion

This is the first study to determine the occurrence of fungal trunk pathogens on *S. cumini* trees in Iran. According to field observations, yellowing, shoot dieback, and branch and trunk cankers were the main external disease symptoms recorded on this host. Internal wood disease symptoms had higher diversity than those recorded as the external disease symptoms. Eight wood lesion types (irregular wood necrosis, wedge-shaped necrosis, arch-shaped necrosis, brown to black wood streaking, central necrosis, black spots, wood decay, and watery necrosis) were associated with trunk diseases of *S. cumini.* These internal wood lesion types were similar to those described by previous authors for stone fruit trees (Gramaje et al. 2012; Soltaninejad et al. 2017), pome fruit trees (Cloete et al. 2011; Sami et al. 2014), forest and ornamental trees (Hashemi et al. 2017; Kazemzadeh Chakusary et al. 2017), pistachio (Mohammadi et al. 2015), and grapevine (van Niekerk et al. 2011) trunk diseases. Different species often co-occurred in the same symptom type, indicating the complexity of the etiology of symptoms observed.

The most predominant fungal taxa isolated from symptomatic or asymptomatic wood of *S. cumini* in this study belonged to the genus *Phaeoacremonium*. These pathogens are mainly associated with esca and Petri disease of grapevines (Gramaje et al. 2015). Four *Phaeoacremonium* species were associated with different wood lesion types. *Phaeoacremonium* isolates were mainly detected from central necrosis, which is in agreement with other studies on grapevine in Spain (Luque et al. 2009) and stone fruit trees in Iran (Soltaninejad et al. 2017). *P. fraxinopennsylvanicum* was obtained from seven out of eight wood lesion types during this survey. By contrast, *P. viticola* was only isolated from irregular wood discoloration and central necrosis. Previous studies on fungal trunk pathogens have shown that, in general, there is no definite relationship between fungal species and wood lesion types (Luque et al. 2009; Mohammadi et al. 2014). All four species of *Phaeoacremonium* have previously been reported from various woody hosts in Iran (Hashemi et al. 2017; Kazemzadeh Chakusary et al. 2017; Mohammadi et al. 2013; Sami et al. 2014) and other countries (Gramaje et al. 2015; Marin-Felix et al. 2018; Mostert et al. 2006; Spies et al. 2018); however, they are reported here for the first time on *S. cumini.*

P. fraxinopennsylvanicum was the unique species isolated from asymptomatic wood samples. Similar results were obtained by Halleen et al. (2003) and Aroca et al. (2010) in South Africa and Spain, respectively, who isolated some grapevine fungal trunk pathogens including *Phaeoacremonium* spp. from asymptomatic rootstock propagation material. In a study conducted by Faraji and Mohammadi (2015), *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora* were isolated from asymptomatic and diseased vines in Yazd province (Iran). Several studies have suggested that *Phaeoacremonium* spp. and other trunk disease pathogens have an endophytic phase (González and Tello 2011; Slippers and Wingfield 2007) and may become pathogenic to grapevine after different abiotic and/or biotic stress conditions; thus, they have been considered as latent pathogens in grapevine (Ferreira et al. 1999). Future research is needed to unravel what triggers latent trunk disease pathogens to transition from an endophyte to a pathogen and cause disease symptoms in grapevine and other hosts.

Botryosphaeriaceae species were the second most predominant fungi associated with cankers and wedge-shaped necrosis of *S. cumini* in this study and included *N. hyalinum*, *D. sapinea*, and *D. seriata.* Our results support similar studies conducted on other

woody hosts such as grapevine (Luque et al. 2009; Úrbez-Torres and Gubler 2009; van Niekerk et al. 2011), *Ulmus carpinifolia* (Hashemi et al. 2017), date palm (Mohammadi 2014), cypress (Mohammadi et al. 2014), and pome (Cloete et al. 2011) and stone fruit trees (Gramaje et al. 2012; Soltaninejad et al. 2017). *N. hyalinum* is cosmopolitan (distributed worldwide) and has been isolated and reported from various plants (Phillips et al. 2013). *D sapinea* is an important pathogen and causes known diseases such as needle and shoot blight, cankers, and dieback on *Pinus* spp. in various countries (Phillips et al. 2013). In Iran, *D. sapinea* has been isolated from pine wood debris in the north of Iran (Kazemzadeh Chakusary et al. 2017). Some species of Botryosphaeriaceae have previously been reported from *Syzygium* spp., including *Neofusicoccum umdonicola*, *N. australe*, *N. cordaticola*, *N. kwambonambiense*, *N. grevilleae*, *N. luteum*, and *Lasiodiplodia gonubiensis* from *Syzygium cordatum* (Pavlic et al. 2004, 2007, 2009), and *N. parvum* from *S. cordatum* (Pavlic et al. 2007) and *Syzygium paniculatum* (Ploetz et al. 2009). Therefore, this study provides the first record of *N. hyalinum*, *D. seriata*, and *D. sapinea* on *S. cumini*.

N. hyalinum was the most dominant species isolated in this study. *N. hyalinum* and *D. seriata* were isolated from both necrotic and asymptomatic wood tissue, whereas *D. sapinea* was only isolated from asymptomatic tissues. *D. sapinea* has been reported as an endophyte and latent pathogen on symptomless pine trees (Bihon et al. 2011). Although many species of the Botryosphaeriaceae family have been considered as endophytes of plants and occur asymptomatically on various woody trees (Jami et al. 2013; Slippers and Wingfield 2007), many are considered important pathogens in several crops. In addition, some Botryosphaeriaceae species have also been found in both asymptomatic and discolored wood tissues, such as *Lasiodiplodia margaritacea* in boab (*Adansonia gregorii*) trees in northwestern Australia (Pavlic et al. 2008), and *Dothiorella viticola* from *Acacia karroo* trees in South Africa (Jami et al. 2013). Unfavorable or stress conditions have also been associated with symptom expression in woody plants with latent infections of Botryosphaeriaceae species (Slippers and Wingfield 2007; Smith et al. 1996; van Niekerk et al. 2011).

Additional trunk pathogens isolated in low numbers in our study include *C. luteo-olivacea* and *Pl. richardsiae*. Both species were associated with irregular wood discoloration and central necrotic wood tissues of *S. cumini. C. luteo-olivacea* has been isolated from symptomatic (Abreo et al. 2008; Gramaje et al. 2011, 2014; Travadon et al. 2015; Úrbez-Torres et al. 2014) and asymptomatic (Casieri et al. 2009; Halleen et al. 2007) wood tissues of grapevine. This fungus had previously been reported from citrus trees in Iran (Espargham 2016), and this study represents the first report of *C. luteo-olivacea* on *S. cumini* in the world. *Pl. richardsiae* is considered a vascular pathogen of grapevine in California (Rolshausen et al. 2010), Italy

Table 2. Number and locality of fungal isolates recovered from symptomatic and asymptomatic wood tissues of *Syzygium cumini*[z]

Isolates			Number of isolates										
				Tissue		Internal wood lesion types							
Family	Species	Location	Total	H	D	IWN	ARN	WSN	WN	CN	WD	BS	BWS
Togniniaceae	*Phaeoacremonium parasiticum*	Kahnouj, Boluk	5	…	5	1	…	…	…	1	…	3	…
	P. viticola	Rudan	3	…	3	1	…	…	…	2	…	…	…
	P. krajdenii	Kahnouj	7	…	7	1	…	…	…	3	…	2	1
	P. fraxinopennsylvanicum	Kahnouj, Boluk, Rudan	19	4	15	2	1	2	1	4	…	3	2
Botryosphaeriaceae	*Neoscytalidium hyalinum*	Kahnouj, Rudan	22	5	17	4	2	6	1	2	…	…	2
	Diplodia sapinea	Rudan	2	2	…	…	…	…	…	…	…		
	D. seriata	Rudan	7	4	3	1		2	…	-	…	…	…
Pleurostomataceae	*Pleurostoma richardsiae*	Kahnouj, Rudan	4	…	4	3	…	…	…	1	…	…	…
	Cadophora luteo-olivacea	Rudan	3	…	3	2	…	…	…	1	…	…	…
Total			72	15	57	15	3	10	2	14	0	8	5

[z] H = from healthy tissues, and D = from diseased tissues. Various internal wood lesion types from which isolates were taken in cross section of affected wood samples: ARN = arch-shaped necrosis; BS = black spots; BWS = brown to black wood streaking; CN = central necrosis; IWN = irregular wood necrosis; WN = watery necrosis; WD = wood decay; and WSN = wedge-shaped necrosis.

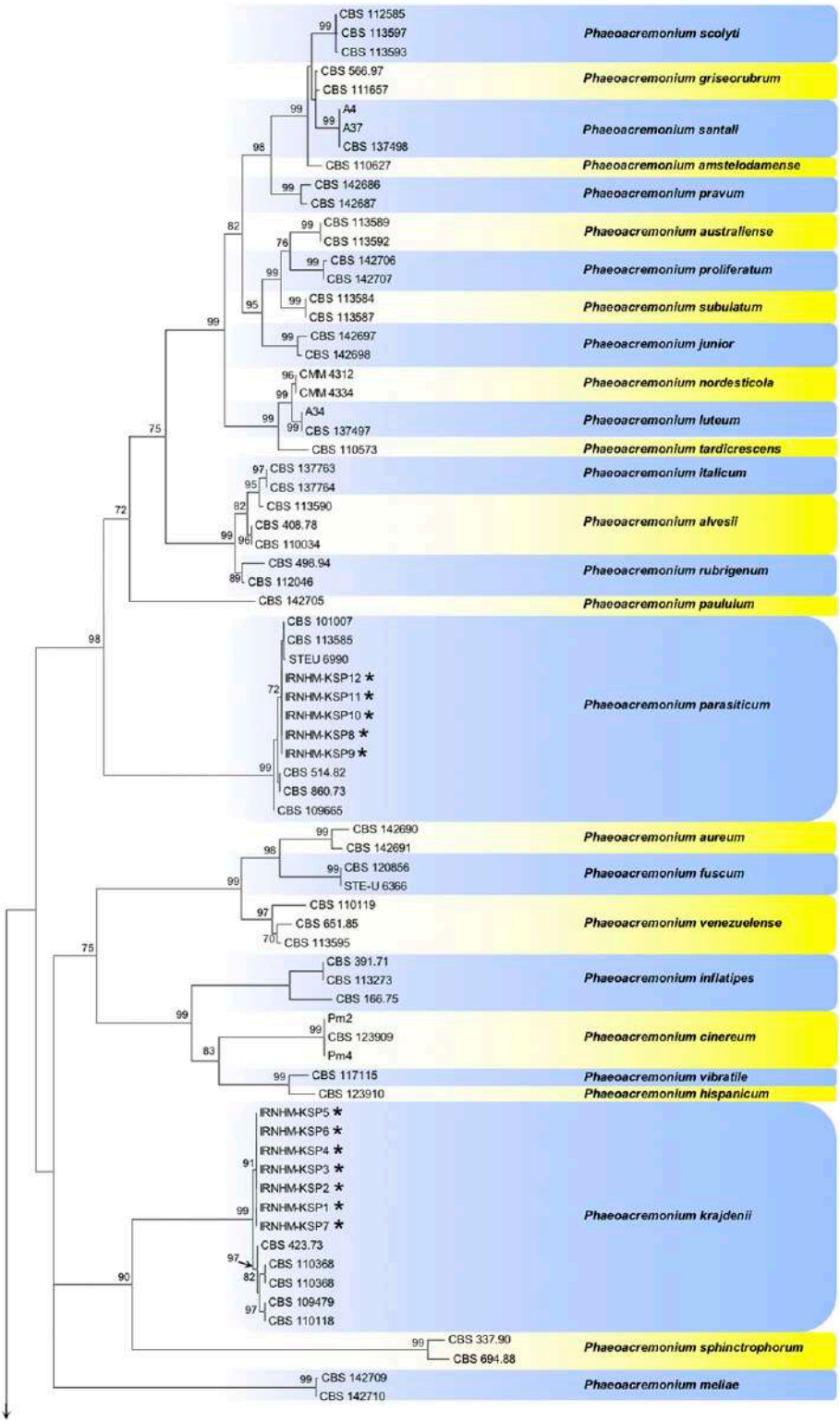
Table 3. Percentage homology (ITS and TEF gene) of some representative isolates obtained from *Syzygium cumini* with related references in GenBank[z]

Isolates in this study		Closest match references in GenBank			
Code	GenBank number	Species	Isolation source	% Homology and code	Reference
IRNHM-KSP35	TEF: MH271386	*Neoscytalidium hyalinum*	*Homo sapiens*	100% with CBS 145.78	Phillips et al. (2013)
	ITS: MH271392	*N. hyalinum*	*Mangifera indica*	99% with CBS 499.66	Phillips et al. (2013)
IRNHM-KSP40	TEF: MH271387	*N. hyalinum*	Sole of foot	100% with CBS 145.78	Phillips et al. (2013)
	ITS: MH271393	*N. hyalinum*	*Mangifera indica*	99% with CBS 499.66	Phillips et al. (2013)
IRNHM-KSP57	TEF: MH271388	*Diplodia sapinea*	*Pinus nigra*	99% with CBS 393.84	Alves et al. (2006)
			Pinus patula	99% with CBS 109725	Alves et al. (2006)
	ITS: MH271394	*D. sapinea*	*Pinus nigra*	100% with CBS 393.84	Alves et al. (2006)
IRNHM-KSP58	TEF MH271389	*D. sapinea*	*Pinus nigra*	99% with CBS 393.84	Alves et al. (2006)
			Pinus patula	99% with CBS 109725	Alves et al. (2006)
	ITS: MH271395	*D. sapinea*	*Pinus nigra*	100% with CBS 393.84	Alves et al. (2006)
IRNHM-KSP59	TEF: MH271390	*D. seriata*	*Vitis vinifera*	100% with CBS 112555	Phillips et al. (2005)
	ITS: MH271396	*D. seriata*	*Vitis* sp.	100% with CBS 119049	Alves et al. (2006)
IRNHM-KSP60	TEF: MH271391	*D. seriata*	*Vitis vinifera*	100% with CBS 112555	Phillips et al. (2005)
	ITS: MH271397	*D. seriata*	*Vitis* sp.	100% with CBS 119049	Alves et al. (2006)
IRNHM-KSP61	ITS: MH271398	*Cadophora luteo-olivacea*	*Citrus reticulata*	100% with7R38-4	Blanchette et al. (2010)
IRNHM-KSP62	ITS: MH271399	*C. luteo-olivacea*	*Citrus reticulata*	100% with 7R38-4	Blanchette et al. (2010)
IRNHM-KSP63	ITS: MH271400	*Pleurostoma richardsiae*	…	99% with CBS 270.33	Vijaykrishna et al. (2004)
IRNHM-KSP64	ITS: MH271401	*Pl. richardsiae*	…	99% with CBS 270.33	Vijaykrishna et al. (2004)

[z] ITS = internal transcribed spacer, and TEF = translation elongation factor 1-α.

(Carlucci et al. 2015), and South Africa (White et al. 2011). This fungus has also been isolated from brown streaking internal wood symptoms of trunks, branches, and twigs of olive trees in Southern Italy (Carlucci et al. 2013). This study also represents the first report of *Pl. richardsiae* on *S. cumini* worldwide.

Results of the pathogenicity tests show significant differences in the degree of virulence among fungal species inoculated to *S. cumini* shoots. Of the nine selected species, the lesions caused by *N. hyalinum* were longer than those caused by the other species. Several studies have proven pathogenicity of *N. hyalinum* on various woody hosts, including *Citrus* spp. (Mayorquin et al. 2016; Polizzi et al. 2011), walnut (Chen et al. 2013), grapevine (Rolshausen et al. 2013), elm (Hashemi et al. 2017), fig (Ray et al. 2010), and pitaya (Chuang et al. 2012). *D. seriata* produced the shortest lesions, and lesions produced by one isolate of this species did not differ significantly from the control. Similar results were reported by



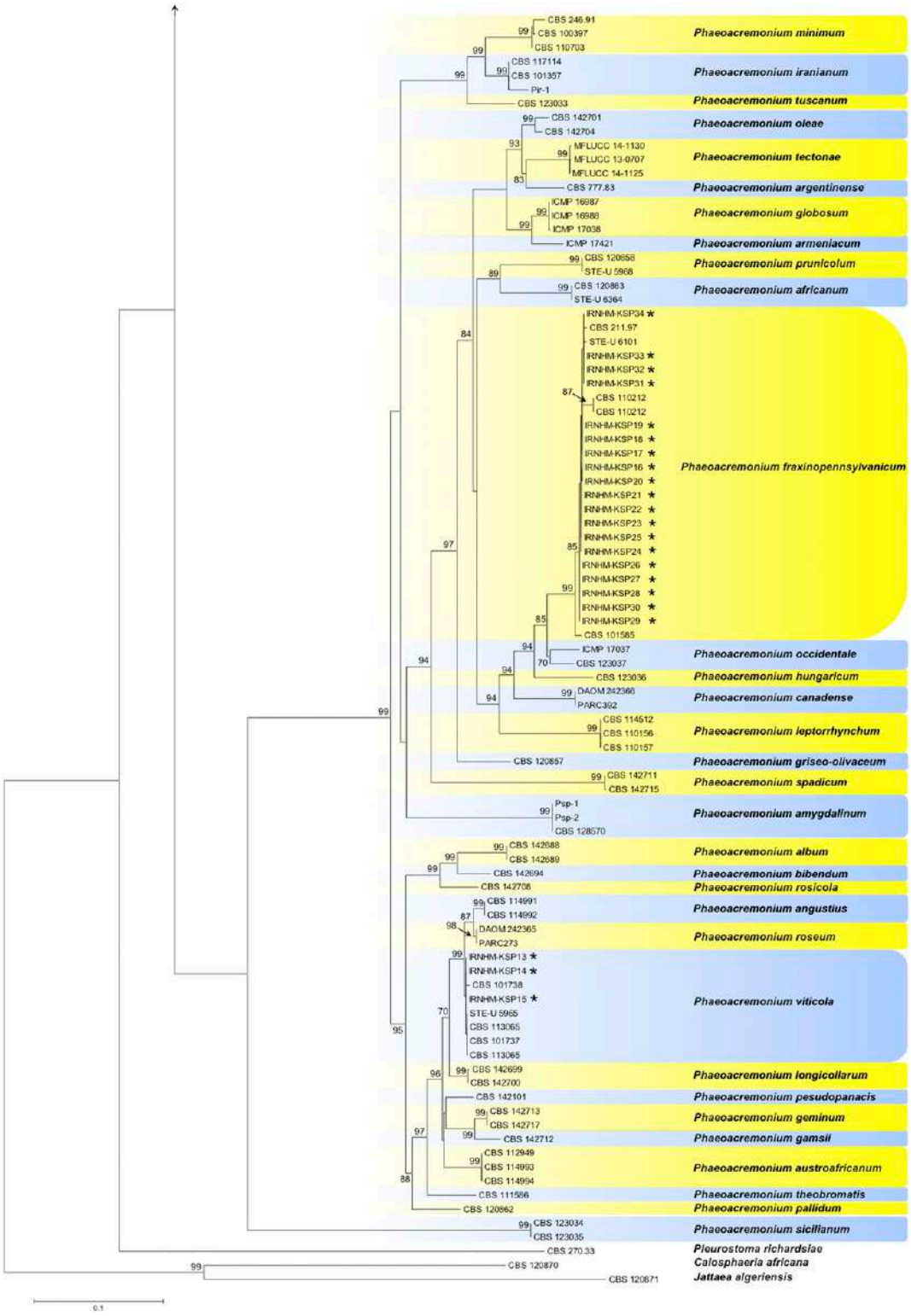
(*Continued*)

Fig. 2. Maximum likelihood phylogeny of the genus *Phaeoacremonium* as estimated from concatenated alignments of the actin and β-tubulin regions. Maximum likelihood bootstrap percentages are indicated at the nodes. Support values less than 70% bootstrap are omitted. Strains collected in this study are indicated by asterisks.

Mohammadi et al. (2013) on grapevine in Iran. Based on host plant, location, and inoculation methods, there have been conflicting reports on the pathogenicity of *D. seriata* in the world. Some researchers considered it to be a primary and virulent pathogen on grapevine (Savocchia et al. 2007; van Niekerk et al. 2004) and apple trees (Brown-Rytlewski and McManus 2000). This fungus was considered a weak pathogen on some woody plants such as grapevine (Úrbez-Torres and Gubler 2009), olive (Moral et al. 2010), walnut (Chen et al. 2014), and poplar trees (Hashemi and Mohammadi 2016). *Pl. richardsiae* and *C. luteo-olivacea* were also pathogenic on *S. cumini* shoots because they caused wood lesions significantly longer than control treatments. In artificial inoculations, *C. luteo-olivacea* has been shown to produce lesions in the xylem of grapevine in Italy (Carlucci et al. 2015), Spain (Gramaje et al. 2011, 2014), and South Africa (Halleen et al. 2007). Carlucci et al. (2013) considered *Pl. richardsiae* to be the most aggressive pathogen among several other fungi isolated from olive trees in Italy.

This work represents the first report of some known fungal trunk pathogens and their pathogenicity on *S. cumini* in Iran and discusses the endophytic character of some of them isolated from asymptomatic wood on this host. Moreover, as mentioned above, most isolated fungal species are reported for the first time on *S. cumini* worldwide.

Fig. 2. (*Continued from previous page*)



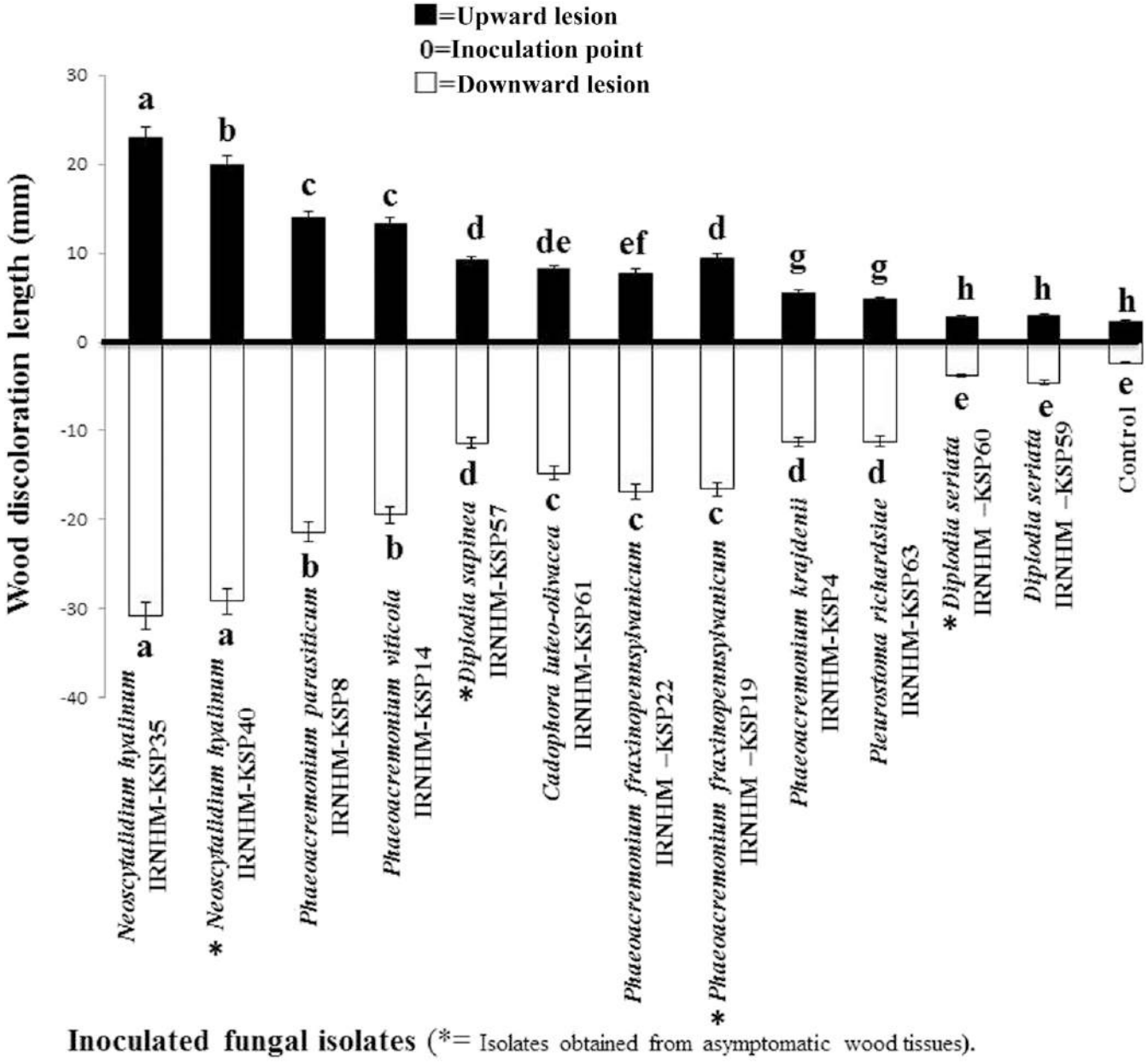


Fig. 3. Upward and downward lesion size produced by inoculated isolates on *Syzygium cumini* shoots, 45 days after inoculation. Bars represent standard error of the means.

Table 4. Pathogenicity and reisolation frequencies of fungal isolates inoculated onto detached *Syzygium cumini* woody shoots 45 days after inoculation[y]

Isolates			
Species	**Accession number**	**Mean lesion length (mm) ± SE**	**Reisolation frequency (%)**
Neoscytalidium hyalinum	IRNHM-KSP35	53.75 ± 0.92 a	94.4
N. hyalinum	IRNHM-KSP40[z]	49.13 b	83.3
Diplodia sapinea	IRNHM-KSP57[z]	20.50 ± 0.87 f	77.8
D. seriata	IRNHM-KSP59	6.50 ± 0.42 hi	61.1
D. seriata	IRNHM-KSP60[z]	7.25 h	38.9
Phaeoacremonium parasiticum	IRNHM-KSP8	35.38 ± 1.05 c	83.3
P. krajdenii	IRNHM-KSP4	16.75 ± 0.88 g	44.4
P. viticola	IRNHM-KSP14	32.88 ± 0.79 d	61.1
P. fraxinopennsylvanicum	IRNHM-KSP19[z]	24.63 ± 0.74 e	88.9
P. fraxinopennsylvanicum	IRNHM-KSP22	24.88 e	61.1
Pleurostoma richardsiae	IRNHM-KSP63	15.88 ± 0.77 g	38.9
Cadophora luteo-olivacea	IRNHM-KSP61	22.88 ± 0.88 de	55.6
Potato dextrose agar plug	–	4.63 ± 0.38 i	0
LSD ($P < 0.05$)		2.287	

[y] Means followed by the same letter are not significantly different (at $P < 0.05$). LSD = least significant difference.
[z] Isolates obtained from healthy (asymptomatic) wood tissues of *S. cumini*.

Therefore, further studies on this host in other regions of Iran and abroad where *S. cumini* is grown will certainly provide important information about fungal trunk pathogens on this plant.

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Literature Cited

Abreo, E., Lupo, S., Martínez, I., and Bettucci, L. 2008. Fungal species associated to grapevine trunk diseases in Uruguay. J. Plant Pathol. 90:591.

Alves, A., Correia, A., and Phillips, A. J. L. 2006. Multiple gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. Fungal Divers. 23:1-15.

Aroca, A., Gramaje, D., Armengol, J., García-Jiménez, J., and Raposo, R. 2010. Evaluation of the grapevine nursery propagation process as a source of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* and occurrence of trunk disease pathogens in rootstock mother vines in Spain. Eur. J. Plant Pathol. 126:165-174.

Bihon, W., Slippers, B., Burgess, T., Wingfield, M. J., and Wingfield, B. D. 2011. Sources of *Diplodia pinea* endophytic infections in *Pinus patula* and *P. radiata* seedlings in South Africa. For. Pathol. 41:370-375.

Blanchette, R. A., Held, B. W., Arenz, B. E., Jurgens, J. A., Baltes, N. J., Duncan, S. M., and Farrell, R. L. 2010. An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. Microb. Ecol. 60:29-38.

Brown-Rytlewski, D. E., and McManus, P. S. 2000. Virulence of *Botryosphaeria dothidea* and *Botryosphaeria obtusa* on apple and management of stem cankers with fungicides. Plant Dis. 84:1031-1037.

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

Carlucci, A., Cibelli, F., Lops, F., Phillips, A. J. L., Ciccarone, C., and Raimondo, M. L. 2015. *Pleurostomophora richardsiae* associated with trunk diseases of grapevines in southern Italy. Phytopathol. Mediterr. 54:109-123.

Carlucci, A., Raimondo, M. L., Cibelli, F., Phillips, A. J. L., and Lops, F. 2013. *Pleurostomophora richardsiae, Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in southern Italy. Phytopathol. Mediterr. 52:517-527.

Casieri, L., Hofstett, V., Viret, O., and Gindro, K. 2009. Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. Phytopathol. Mediterr. 48:73-83.

Chen, S. E., Gryzenhout, M., Roux, J., Xie, Y. J., Wingfield, M. J., and Zhou, X. D. 2010. Identification and pathogenicity of *Chrysoporthe cubensis* on *Eucalyptus* and *Syzygium* spp. in South China. Plant Dis. 94:1143-1150.

Chen, S. F., Fichtner, E., Morgan, D. P., and Michailides, T. J. 2013. First report of *Lasiodiplodia citricola* and *Neoscytalidium hyalinum* causing death of graft union of English walnut in California. Plant Dis. 97:993.

Chen, S. F., Morgan, D. P., Hasey, J. K., Anderson, K., and Michailides, T. J. 2014. Phylogeny, morphology, distribution, and pathogenicity of Botryosphaeriaceae and Diaporthaceae from English walnut in California. Plant Dis. 98:636-652.

Chuang, M. F., Ni, H. F., Yang, H. R., Shu, S. L., Lai, S. Y., and Jiang, Y.-L. 2012. First report of stem canker disease of pitaya (*Hylocereus undatus* and *Hylocereus polyrhizus*) caused by *Neoscytalidium dimidiatum* in Taiwan. Plant Dis. 96:906.

Cloete, M., Fourie, P. H., Damm, U., Crous, P. W., and Mostert, L. 2011. Fungi associated with die-back symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease pathogens. Phytopathol. Mediterr. 50(Suppl.):S176-S190.

Craven, L. A., and Biffin, E. 2010. An infrageneric classification of *Syzygium* (Myrtaceae). Blumea 55:94-99.

Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. 2012. JModelTest 2: More models, new heuristics and parallel computing. Nat. Methods 9:772.

Espargham, N. 2016. Isolation and identification of fungi associated with yellowing, dieback and decline of citrus in Bushehr province. Master's thesis. Shahid Bahonar University of Kerman, Iran.

Faraji, F., and Mohammadi, H. 2015. Endophytic fungi associated with healthy and diseased wood tissues of grapevines in Yazd province. 2nd Iranian Mycological Congress, 23–25 August 2015, University of Tehran, Karaj, Iran.

Ferreira, J. H. S., van Wyk, P. S., and Calitz, F. J. 1999. Slow dieback of grapevine in South Africa: Stress-related predisposition of young vines for infection by *Phaeoacremonium chlamydosporum*. S. Afr. J. Enol. Vitic. 20:43-46.

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

González, V., and Tello, M. 2011. The endophytic mycota associated with *Vitis vinifera* in central Spain. Fungal Divers. 47:29e42.

Gramaje, D., Agustí-Brisach, C., Pérez-Sierra, A., Moralejo, E., Olmo, D., Mostert, L., Damm, U., and Armengol, J. 2012. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). Persoonia 28:1-13.

Gramaje, D., Baumgartner, K., Halleen, F., Mostert, L., Sosnowski, M. R., Úrbez-Torres, J. R., and Armengol, J. 2016. Fungal trunk diseases: A problem beyond grapevines? Plant Pathol. 65:355-356.

Gramaje, D., León, M., Santana, M., Crous, P. W., and Armengol, J. 2014. Multilocus ISSR markers reveal two major genetic groups in Spanish and South African populations of the grapevine fungal pathogen *Cadophora luteo-olivacea*. PLoS One 9:e110417.

Gramaje, D., Mostert, L., and Armengol, J. 2011. Characterization of *Cadophora luteo-olivacea* and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine nurseries in Spain. Phytopathol. Mediterr. 50:112-126.

Gramaje, D., Mostert, L., Groenewald, J. Z., and Crous, P. W. 2015. *Phaeoacremonium*: From esca disease to phaeohyphomycosis. Fungal Biol. 119:759-783.

Halleen, F., Crous, P. W., and Petrini, O. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Australas. Plant Pathol. 32:47-52.

Halleen, F., Mostert, L., and Crous, P. W. 2007. Pathogenicity testing of lesser-known vascular fungi of grapevines. Australas. Plant Pathol. 36:277-285.

Harrington, T. C., and McNew, D. L. 2003. Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the Helotiales. Mycotaxon 87:141-151.

Hashemi, H., and Mohammadi, H. 2016. Identification and characterization of fungi associated with internal wood lesions and decline disease of willow and poplar trees in Iran. For. Pathol. 46:341-352.

Hashemi, H., Mohammadi, H., and Abdollahzadeh, J. 2017. Symptoms and fungi associated with elm trees decline in Iran. Eur. J. For. Res. 136:857-879.

Heath, R. N., Gryzenhout, M., Roux, J., and Wingfield, M. J. 2006. Discovery of *Chrysoporthe cubensis* on native *Syzygium* species in South Africa. Plant Dis. 90:433-438.

Jami, F., Slippers, B., Wingfield, M. J., and Gryzenhout, M. 2013. Greater Botryosphaeriaceae diversity in healthy than associated diseased *Acacia karroo* tree tissues. Australas. Plant Pathol. 42:421-430.

Jankowiak, R., Stepniewska, H., Szwagrzyk, J., Bilański, P., and Gratzer, G. 2016. Characterization of *Cylindrocarpon*-like species associated with litter in the old-growth beech forests of Central Europe. For. Pathol. 46:582-594.

Katoh, K., and Toh, H. 2010. Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics 26:1899-1900.

Kazemzadeh Chakusary, M., Mohammadi, H., and Khodaparast, A. 2017. Decline-associated *Phaeoacremonium* species occurring on forest trees in the north of Iran. For. Pathol. 47:e12368.

Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870-1874.

Luque, J., Martos, S., Aroca, A., Raposo, R., and Garcia- Fihueres, F. 2009. Symptoms and fungi associated with declining mature grapevine plants in northeast Spain. J. Plant Pathol. 91:381-390.

Marin-Felix, Y., Hernández-Restrepo, M., Wingfield, M. J., Akulov, A., Carnegie, A. J., Cheewangkoon, R., Gramaje, D., Groenewald, J. Z., Guarnaccia, V., Halleen, F., Lombard, L., Luangsa-ard, J., Marincowitz, S., Moslemi, A., Mostert, L., Quaedvlieg, W., Schumacher, R. K., Spies, C. F. J., Thangavel, R., Taylor, P. W. J., Wilson, A. M., Wingfield, B. D., Wood, A. R., and Crous, P. W. 2018. Genera of phytopathogenic fungi: GOPHY 2. Stud. Mycol. 92:47-133.

Mayorquin, J. S., Wang, D. H., Twizeyimana, M., and Eskalen, A. 2016. Identification, distribution, and pathogenicity of Diatrypaceae and Botryosphaeriaceae associated with citrus branch canker in the southern California desert. Plant Dis. 100:2402-2413.

Modi, D. C., Patel, J. K., Shah, B. N., and Nayak, B. S. 2010. Pharmacognostic studies of the seed of *Syzygium cumini* Linn. Pharma Sci. Monitor 1:20-26.

Mohammadi, H. 2014. *Phaeoacremonium* spp. and Botryosphaeriaceae spp. associated with date palm (*Phoenix dactylifera* L.) decline in Iran. J. Phytopathol. 162:575-581.

Mohammadi, H., Banihashemi, Z., Gramaje, D., and Armengol, J. 2013. Characterization of *Diplodia seriata* and *Neofusicoccum parvum* associated with grapevine decline in Iran. J. Agric. Sci. Technol. 15:603-616.

Mohammadi, H., Kazemi, S., and Farahmand, H. 2014. *Phaeoacremonium* and Botryosphaeriaceae species associated with cypress (*Cupressus sempervirens* L.) decline in Kerman province (Iran). Phytopathol. Mediterr. 51:27-39.

Mohammadi, H., Sarcheshmehpour, M., and Mafi, E. 2015. Fungal trunk pathogens associated with wood decay of pistachio trees in Iran. Span. J. Agric. Res. 13:e1007.

Mora-Sala, B., Cabral, C., León, M., Agustí-Brisach, C., Armengol, J., and Abad-Campos, P. 2018. Survey, identification and characterization of *Cylindrocarpon*-like asexual morphs in Spanish forest nurseries. Plant Dis. 102:2083-2100.

Moral, J., Muñoz-Díez, C., González, N., Trapero, A., and Michailides, T. J. 2010. Characterization and pathogenicity of Botryosphaeriaceae species collected from olives and other hosts in Spain and California. Phytopathology 100: 1340-1351.

Mostert, L., Groenewald, J. Z., Summerbell, R. C., Gams, W., and Crous, P. W. 2006. Taxonomy and pathology of *Togninia* (Diaporthales) and its *Phaeoacremonium* anamorphs. Stud. Mycol. 54:1-113.

O'Donnell, K., and Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are non-orthologous. Mol. Phylogenet. Evol. 7:103-116.

Pavlic, D., Slippers, B., Coutinho, T. A., Gryzenhout, M., and Wingfield, M. J. 2004. *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. Stud. Mycol. 50:313-322.

Pavlic, D., Slippers, B., Coutinho, T. A., and Wingfield, M. J. 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. Plant Pathol. 56:624-636.

Pavlic, D., Slippers, B., Coutinho, T. A., and Wingfield, M. J. 2009. Molecular and phenotypic characterization of three phylogenetic species discovered within the *Neofusicoccum parvum/N. ribis* complex. Mycologia 101:636-647.

Pavlic, D., Wingfield, M. J., Barber, P., Slippers, B., Hardy, G., and Burgess, T. I. 2008. Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia. Mycologia 100:851-866.

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

Phillips, A., Alves, A., Correia, A., and Luque, J. 2005. Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. Mycologia 97:513-529.

Phillips, A. J. L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M. J., Groenewald, J. Z., and Crous, P. W. 2013. The Botryosphaeriaceae: Genera and species known from culture. Stud. Mycol. 76:51-167.

Ploetz, R., Pérez-Martínez, J., Palmateer, A., and Cating, R. 2009. *Neofusicoccum parvum* causes a lethal dieback of *Syzygium paniculatum* in Florida. Plant Pathol. 58:801.

Polizzi, G., Aiello, D., Castello, I., and Vitale, A. 2011. Occurrence, molecular characterisation, and pathogenicity of *Neoscytalidium dimidiatum* on citrus in Italy. Acta Hortic. 892:237-243.

Prabhakaran, S., Gothandam, K. M., and Sivashanmugam, K. 2011. Phytochemical and antimicrobial properties of *Syzygium cumini* an ethanomedicinal plant of Javadhu hills. Res. Pharm. 1:22-33.

Ray, J. D., Burgess, T., and Lanoiselet, V. M. 2010. First record of *Neoscytalidium dimidiatum* and *N. novaehollandiae* on *Mangifera indica* and *N. dimidiatum* on *Ficus carica* in Australia. Australas. Plant Dis. Notes 5:48-50.

Réblová, M., Jaklitsch, W. M., Réblová, K., and Štìpánek, V. 2015. Phylogenetic reconstruction of the *Calosphaeriales* and *Togniniales* using five genes and predicted RNA secondary structures of ITS, and *Flabellascus tenuirostris* gen. et sp. nov. PLoS One 10:e0144616.

Rolshausen, P. E., Akgul, D. S., Perez, R., Eskalen, A., and Gispert, C. 2013. First report of wood canker caused by *Neoscytalidium hyalinum* on grapevine in California. Plant Dis. 97:1511.

Rolshausen, P. E., Úrbez-Torres, J. R., Rooney-Latham, S., Eskalen, A., Smith, R. J., and Gubler, W. D. 2010. Evaluation of pruning wound susceptibility and protection against fungi associated with grapevine trunk diseases. Am. J. Enol. Vitic. 61:113-119.

Sami, S., Mohammadi, H., and Heydarnejad, J. 2014. *Phaeoacremonium* species associated with necrotic wood of pome fruit trees in Iran. J. Plant Pathol. 96:19-27.

Savocchia, S., Steel, C. C., Stodart, B. J., and Somers, A. 2007. Pathogenicity of *Botryosphaeria* species isolated from declining grapevines in subtropical regions of Eastern Australia. Vitis 46:27-32.

Slippers, B., Fourie, G., Crous, P. W., Coutinho, T. A., Wingfield, B. D., Carnegie, A. J., and Wingfield, M. J. 2004. Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. Stud. Mycol. 50:343-358.

Slippers, B., and Wingfield, M. J. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. Fungal Biol. Rev. 21:90-106.

Smith, H., Wingfield, M. J., and Petrini, O. 1996. *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. For. Ecol. Manage. 89:189-195.

Soltaninejad, N., Mohammadi, H., and Massumi, H. 2017. Isolation, identification and pathogenicity of Botryosphaeriaceae and *Phaeoacremonium* species associated with decline of *Prunus* species in Iran. J. Plant Pathol. 99:571-581.

Spies, C. F. J., Moyo, P., Halleen, F., and Mostert, L. 2018. *Phaeoacremonium* species diversity on woody hosts in the Western Cape Province of South Africa. Persoonia 40:26-62.

Swofford, D. L. 2003. PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10. http://paup.phylosolutions.com/documentation/faq/.

Travadon, R., Lawrence, D. P., Rooney-Latham, S., Gubler, W. D., Wilcox, W. F., Rolshausen, P. E., and Baumgartner, K. 2015. *Cadophora* species associated with wood-decay of grapevine in North America. Fungal Biol. 119:53-66.

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

Úrbez-Torres, J. R., Haag, P., Bowen, P., and O'Gorman, D. T. 2014. Grapevine trunk diseases in British Columbia: Incidence and characterization of the fungal pathogens associated with esca and Petri diseases of grapevine. Plant Dis. 98: 456-468.

van Niekerk, J. M., Bester, W., Halleen, F., Crous, P. W., and Fourie, P. H. 2011. The distribution and symptomatology of grapevine trunk disease pathogens are influenced by climate. Phytopathol. Mediterr. 50:S98-S111.

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

Vijaykrishna, D., Mostert, L., Jeewon, R., Gams, W., Hyde, K. W., and Crous, P. W. 2004. *Pleurostomophora*, an anamorph of *Pleurostoma* (Calosphaeriales), a new anamorph genus morphologically similar to *Phialophora*. Stud. Mycol. 50: 387-395.

White, C. L., Halleen, F., Fischer, M., and Mostert, L. 2011. Characterisation of the fungi associated with esca diseased grapevines in South Africa. Phytopathol. Mediterr. 50:S204-S223.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and J. White, eds. Academic Press, San Diego, CA.