Fungal pathogens associated with black foot of grapevine in China

Qingtong YE1,2, Wei ZHANG1, Jingyi JIA1, Xinghong LI1, Yueyan ZHOU1,2, Changping HAN1,2, Xuehong WU2, Jiye YAN1,*

1 Beijing Key Laboratory of Environment Friendly Management on Fruit Diseases and Pests in North China, Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
2 Department of Plant Pathology, China Agricultural University, Beijing 100193, China
*Corresponding author. E-mail: jiyeyan@vip.163.com

Summary. Grapevine trunk diseases (GTDs) are the most destructive diseases in grape-growing regions worldwide. Black foot is one of the important GTDs affecting young vineyards and nurseries. This disease has not been reported in China. During 2017 and 2019, field surveys were carried out in the Guangxi, Hebei, Ningxia, Shanxi, and Xinjiang provinces of China. Incidence of plants with black foot symptoms was 0.1% to 1% in the surveyed vineyards. Plant samples with poorly developed shoots and canes, chlorotic leaves, and necrotic trunks or roots were collected from the five provinces. In total, 50 fungal isolates were obtained from symptomatic tissues. Based on morphological and multi-gene phylogenetic analyses, five species were identified as Cylindrocladiella lageniformis, Dactylonectria torresensis, D. macrodidyma, D. alcacerensis, and Neonectria sp.1. Pathogenicity was assessed using young, healthy detached green shoots of grapevine ‘Summer Black’ and potted 3-month-old ‘Summer Black’ cuttings. Fungi were re-isolated from necrotic lesions. Among the five species, D. macrodidyma was the most aggressive. This is the first report of C. lageniformis, D. torresensis, D. macrodidyma, D. alcacerensis, and Neonectria sp.1 associated with black foot in China. This study has enhanced knowledge of the fungi associated with black foot in China, and will assist development of control measures for this disease.

Keywords. Nectriaceae, morphological characteristics, phylogenetic analyses, Vitis vinifera.

INTRODUCTION

Grapevine (Vitis vinifera L.) is an economically important fruit crop, with global cultivation area of 7,449,000 hectares in 2018, and China is ranked the second in the world grapevine cultivation area (2019 OIV). More than 70 diseases have been reported in grapevines, most of which are caused by fungi or oomycetes (Wilcox et al., 2006), and among these, at least 27 diseases have been reported in China. Esca complex, Botryosphaeria dieback, black foot (BF), Eutypa dieback, and Phomopsis dieback are major fungal
grapevine trunk diseases (GTDs) worldwide. These diseases have been reported in almost all the main grape-growing countries (Gramaje et al., 2018). GTDs are complexes that affect grape yields, wine quality and lifespan of plants in many grape-growing regions. The global financial losses attributed to GTDs are estimated to be more than $US 1.5 billion per year (Hofstetter et al., 2012).

Black foot (BF) is one of the most significant GTDs, especially in nurseries and young plantations (Halleen et al., 2006). This disease has occurred in many viticulture regions during the last decade, including Australia, Brazil, California, Canada, (British Columbia, Quebec), France, Iran, Italy, New Zealand, Portugal, South Africa (Western Cape), Spain, Switzerland, Turkey, United States of America, and Uruguay (Agustí-Brisach and Armengol, 2013; Lombard et al., 2014; Carlucci et al., 2017; Aigoun-Mouhous et al., 2019; Lawrence et al., 2019; Berlanas et al., 2020). Among these species, *I. lirioidendri* and *D. macrodidyma* are the most widely distributed ones (Agustí-Brisach and Armengol, 2013). These fungi are frequently isolated from BF symptoms in nursery and older grapevine plants (Petit et al., 2011; Carlucci et al., 2017), and from asymptomatic inner tissues from plants (Berlanas et al., 2020). Some BF fungi have also been detected from the soils of grapevine nurseries and vineyards, in Spain and South Africa. (Agustí-Brisach et al., 2013, 2014; Langenhovven et al., 2018).

Eutypa dieback was first reported in China in 2007, Botryosphaeria dieback in 2010, Diaporthe dieback in 2015 and Esca in 2020 (Li et al., 2007; Li et al., 2010; Dissanayake et al., 2015; Ye et al., 2020), while BF has not been reported in China previously.

**MATERIALS AND METHODS**

**Vineyard surveys**

Surveys were carried out in ten vineyards, located in Ningxia, Hebei, Shanxi, Guangxi and Xinjiang provinces of China, during 2017 and 2019 (Figure 1a). These provinces belong to the top ten grape cultivated grape areas in China, and Xinjiang province ranked the first, followed by Hebei province. The training systems used in the surveyed vineyards was mini ”J”. The vineyards were of similar age, from 5 to 6 years old. Typical symptoms associated with diseased vines were shortened shoot internodes, chlorotic leaves, and trunk and root necroses (Figure 1, b–h). Initial disease symptoms included root necroses (especially small roots). As the disease progressed, the above-ground plant parts developed shoot shortened internodes and chlorotic leaves in severe cases. Some grapevines were grafted (rootstock Fercal), and some others were self-rooted (Personal communication, some of the grape growers).

**Sample collection, fungus isolation and morphology of the pathogens**

Samples were collected from *V. rotundifolia* Michx., and *V. vinifera* cvs Marselan, Cabernet Franc or Cabernet Sauvignon. Typical symptoms were recorded by taking appropriate photographs. The samples were kept at 4°C for further study, and the presence of spores or structures on the surfaces of trunks or roots were detected using a microscope. Isolations were made from
symptomatic trunks and roots. Necrotic root and trunk samples were debarked and cut into small pieces (4–5 mm²). These small pieces were then surface-sterilized in 75% ethanol for 30 s, rinsed three times with sterilized water, dried, and cultured on potato dextrose agar (PDA; 20% potatoes, 2% dextrose, 1.5 to 2% agar) in Petri plates. The plates were incubated at 25°C. Fungi growing from tissue pieces were transferred onto new PDA plates after 7 d, and pure cultures were obtained by isolating single spores. Pure cultures were grown on PDA and malt extract agar (MEA) and incubated at 25°C in the dark for 7 d. Conidia and colonies on the MEA plates...
were observed and photographed using the Axio Imager Z2 photographic microscope (Carl Zeiss Microscopy).

**DNA amplification and phylogenetic analyses**

Single-spore purification were done for all the isolates before DNA extractions. Total genomic DNA was extracted from 50–100 mg of mycelium after 14 d of incubation on PDA (Guo et al., 2000). For initial genus identification, the internal transcribed spacer and intervening 5.8S gene regions (ITS) were amplified and sequenced for all the isolates, and the resulting sequences were searched using BLASTN within GenBank/NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi), as described by Manawasinghe et al. (2019). All the isolates in the present study belonged to *Cylindrocladiella*, *Neonectria* or *Dactylonectria*.

For species confirmation, phylogenetic analyses were conducted using multigene phylogenies. For *Cylindrocladiella*, histone H3 (his3), β-tubulin (tub2), and partial translation elongation factor 1-alpha (tef1) were sequenced (Marin-Felix et al., 2019). ITS, tub2, his3, and tef1 gene regions were sequenced for *Dactylonectria* and *Neonectria* species (Berlanas et al., 2020). The primer pairs and amplification protocols used in the present study are summarized in Table 1. Each PCR mixture comprised 1.0 µL of genomic DNA, 0.6 µL of TaKaRa ExTaq DNA polymerase, 5.0 µL of 10 × ExTaq DNA polymerase buffer, 4.0 µL of dNTPs, and 1.0 µL of each primer, and was adjusted with sterilized double-distilled water to a final volume of 50.0 µL. The PCR reactions were carried out in a thermal cycler (Bio-Rad, model C1000). Amplification products were visualized on 1% agarose electrophoresis gels under UV light using a Gel DocTM XR+ Molecular Imager (Bio-Rad). All positive bands obtained by PCR amplification were sequenced by Tsingke Company, Beijing, China, and the sequence data obtained were deposited in GenBank (Table 2).

Reference sequences of related taxa were obtained from GenBank (Marin-Felix et al., 2019; Berlanas et al., 2020). The sequence data generated in the present study were included, and individual gene regions were aligned using the MAFFT v. 7 webserver (Kuraku et al., 2013; Katoh et al., 2019) (https://mafft.cbrc.jp/alignment/server/). The alignments were checked and edited manually, where necessary using BioEdit v7.0.9 (Hall, 1999). Phylogenetic trees were generated using Maximum Likelihood (ML) in RAxML (Silvestro and Michalak, 2016) and Maximum Parsimony (MP) in PAUP (v4.0) (Swofford, 2002). The ML and MP trees were constructed using the methods described by Manawasinghe et al. (2019). For MP, heuristic searches were conducted with 1000 bootstrap replicates by random addition. All characters were unordered and equally weighted. Gaps were treated as missing data, and the steepest descent option not in effect, whereas the MulTrees option was used. The Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC), and Homoplasy Index (HI) were calculated in PAUP. All the resulting trees were saved and checked using Kishino-Hasegawa tests (Kishino and Hasegawa, 1989). The ML analyses of single genes and combined multiple genes were accomplished using the RAxML-HPC2 on XSEDE (8.2.8) in the CIPRES Science Gateway (https://www.phylo.org/portal2/createTask!create.action). Phylogenetic trees were visualized using FigTree v1.4.4 (Rambaut, 2018) and were annotated in Microsoft PowerPoint 2016.

**Pathogenicity tests**

Pathogenicity tests of potential BF pathogens were conducted on detached green shoots or potted 3-month-old tomato seedlings in a greenhouse. All positive bands obtained by PCR amplification were sequenced by Tsingke Company, Beijing, China, and the sequence data obtained were deposited in GenBank (Table 2).

Reference sequences of related taxa were obtained from GenBank (Marin-Felix et al., 2019; Berlanas et al., 2020). The sequence data generated in the present study were included, and individual gene regions were aligned using the MAFFT v. 7 webserver (Kuraku et al., 2013; Katoh et al., 2019) (https://mafft.cbrc.jp/alignment/server/). The alignments were checked and edited manually, where necessary using BioEdit v7.0.9 (Hall, 1999). Phylogenetic trees were generated using Maximum Likelihood (ML) in RAxML (Silvestro and Michalak, 2016) and Maximum Parsimony (MP) in PAUP (v4.0) (Swofford, 2002). The ML and MP trees were constructed using the methods described by Manawasinghe et al. (2019). For MP, heuristic searches were conducted with 1000 bootstrap replicates by random addition. All characters were unordered and equally weighted. Gaps were treated as missing data, and the steepest descent option not in effect, whereas the MulTrees option was used. The Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC), and Homoplasy Index (HI) were calculated in PAUP. All the resulting trees were saved and checked using Kishino-Hasegawa tests (Kishino and Hasegawa, 1989). The ML analyses of single genes and combined multiple genes were accomplished using the RAxML-HPC2 on XSEDE (8.2.8) in the CIPRES Science Gateway (https://www.phylo.org/portal2/createTask!create.action). Phylogenetic trees were visualized using FigTree v1.4.4 (Rambaut, 2018) and were annotated in Microsoft PowerPoint 2016.

**Table 1.** The primer pairs and their amplified protocols used in present study.

| Gene region | Primers | Sequence 5'-3' | Protocols for PCR | References |
|-------------|---------|----------------|-------------------|------------|
| ITS         | ITS1    | TCCGTAGGTTAACACCGGG | 94°C: 3 min, (94°C: 30 s, 52°C: 30 s, 72°C: 7 min) | White et al. (1990) |
|             | ITS4    | TCCGTAGGTTAACACCGGG | 94°C: 3 min, (94°C: 30 s, 52°C: 30 s, 72°C: 7 min) | White et al. (1990) |
| HIS         | CYLH3F  | AGGTTACCTGGTTGCGAG | 94°C: 3 min, (94°C: 30 s, 58°C: 30 s, 72°C: 7 min) | Crous et al. (2004) |
|             | CYLH3R  | AGGTAGGCAGCTGTGGGTAGGA | 94°C: 3 min, (94°C: 30 s, 58°C: 30 s, 72°C: 7 min) | Crous et al. (2004) |
| β-tubulin   | T1      | AGACATCGGCTGAGTTGAGT | 94°C: 3 min, (94°C: 30 s, 58°C: 62°C, 7s, 72°C: 7 min) | Glass and Donaldson (1995) |
|             | Bt2b    | ACCAGCTTGAGTTGAGT | 94°C: 3 min, (94°C: 30 s, 58°C: 30 s, 72°C: 7 min) | Glass and Donaldson (1995) |
| EF1-α       | EF1-72SF | CATCGGAGAGGTCAGAAGGAG | 94°C: 3 min, (94°C: 30 s, 54°C: 30 s, 72°C: 7 min) | Carbone and Kohn (1999) |
|             | EF1-986R | TACTGGAGAGGACCGTTACC | 94°C: 3 min, (94°C: 30 s, 54°C: 30 s, 72°C: 7 min) | Udayanga et al. (2012a, b) |
Table 2. Reference sequence data obtained from GenBank and isolate sequence data from the present study which were used for phylogenetic tests.

| Species          | Isolates | GenBank accession No. |
|------------------|----------|-----------------------|
|                  |          | ITS   | tub2   | his3   | tef1   |
| Neonectria coccinea | CBS 119158 | JF68759 | KC660727 | N/A     | DQ789749.1 |
| N. confusa       | CBS 127484 | KM515889 | KM515886 | N/A     | N/A     |
| N. confusa       | CBS 127485 | JF560437 | JF860054 | N/A     | JF268736.1 |
| N. ditissima     | CBS 226.31  | JF735309 | DQ789869 | JF735594 | JF735783 |
| N. faginata      | CBS 217.67  | JF735310 | DQ789880 | JF735595 | JF735784 |
| N. faginata      | CBS 119160  | HQ840385 | JF268730 | N/A     | JF268746.1 |
| N. fuckeliana    | CBS 119200  | HQ840387 | JF268731 | N/A     | JF268747.1 |
| N. fuckeliana    | CBS 239.29  | HQ840386 | DQ789871 | N/A     | JF268748.1 |
| N. hederae       | CBS 714.97  | N/A     | DQ789878 | N/A     | KC660461 |
| N. hederae       | IMI 058770  | N/A     | DQ789895 | N/A     | DQ789752 |
| N. lugdunensis   | CBS 125475  | KM231762 | KM232019 | KM231482.1 | KM231887.1 |
| N. lugdunensis   | CBS 125485  | KM231762 | KM232019 | KM231482 | KM231887 |
| N. major         | CBS 240.29  | JF735308 | DQ789872 | JF735593 | JF735782 |
| N. neomacrospora | CBS 198.62  | A009255  | HM352865 | KM231481 | HM364351 |
| N. neomacrospora | CBS 324.61  | JF735312 | DQ789875 | JF735599 | JF735788 |
| N. neomacrospora | CBS 503.67  | AY677261 | JF735436 | JF735600 | JF735789 |
| N. obtusispora   | CBS 183.36  | AM419061 | AM419085 | JF735607 | JF735796 |
| N. obtusispora   | CPC 13544   | AY295306 | JF735443 | JF735608 | JF735797 |
| N. panica        | CBS 242.29  | KC660522 | DQ789873 | N/A     | DQ789730 |
| N. panica        | CBS 119724  | KC660496 | DQ789824 | N/A     | DQ789681 |
| N. quercicola    | CBS 143704  | KY676880 | KY676874 | KY676862 | KY676868 |
| N. quercicola    | CPC 13530   | AY295302 | JF735441 | JF735605 | JF735794 |
| N. ramulareae    | MAFF411012  | JX034565.1 | JX034567.1 | N/A     | N/A     |
| N. ramulareae    | CBS 151.29  | AY677291 | JF735438 | JF735602 | JF735792 |
| N. ramulareae    | CBS 182.36  | HM054157 | JF735439 | JF735603 | JF735792 |
| Neonectria sp. 1 | CPC 13545   | N/A     | JF735437 | JF735601 | JF735790 |
| Neonectria sp. 1 | JZB3210004  | MN988722 | MN958534 | MN958545 | MN956387 |
| N. tsugae        | CBS 788.69  | KM231763 | KM232020 | N/A     | DQ789720 |
| Dactylonectria alcalerosis | CBS 129087  | JF735333 | N/A     | JF735630 | JF735919 |
| D. alcalerosis   | Cy134     | JF735332 | N/A     | JF735629 | JF735818 |
| D. alcalerosis   | JZB310007  | MN988716 | MN958528 | MN958539 | MN956381 |
| D. amazonica     | MUCL 55430 | M683706 | M683643  | M683686  | M683664  |
| D. anthuricola   | CBS 564.95 | JF735302 | JF735430 | JF735579 | JF735796.1 |
| D. eucadoriensis | MUCL 55424 | M683704 | M683641  | M683684  | M683662  |
| D. eucadoriensis | MUCL55425  | M683705 | M683642  | M683684  | M683663  |
| D. estremocencis | CPC 13539  | JF735330 | JF735485 | JF735627 | JF735816 |
| D. estremocencis | CBS 129085  | JF735320 | JF735448 | JF735617 | JF735806 |
| D. hispanica     | CBS 142827 | KY676882 | KY676876 | KY76864 | KY676870 |
| D. hispanica     | Cy228     | JF735301 | JF735429 | JF735578 | JF735767 |
| D. hordeicola    | CBS 162.89 | AM419060 | AM419084 | JF735610 | JF735799 |
| D. macrodidyma   | CBS 112601 | M1862898 | AY677229 | JF735644 | JF735833 |
| D. macrodidyma   | CBS 112615 | AY677290 | AY677233 | JF735647 | JF268750 |
| D. macrodidyma   | Cy258     | JF735348 | JF735477 | JF735656 | JF735845 |
| D. macrodidyma   | CBS 112604 | AY677284 | AY677229 | JF735644 | JF735833 |
| D. macrodidyma   | JZB310008  | MN988717 | MN958529 | MN958540 | MN956382 |
| D. macrodidyma   | JZB310009  | MN988718 | MN958530 | MN958541 | MN956383 |

(Continued)
| Species Isolates | GenBank accession No. |
|-----------------|----------------------|
|                 | ITS                  | tub2     | his3     | tef1     |
| **D. macrodidyma** | JZB33100010          | MN988719 | MN958531 | MN958542 | MN956384 |
| **D. novozelandica** | CBS 112608           | AY677288 | AY677235 | JF735632 | JF735821 |
| **D. palmicola**   | CBS 120171           | EF607089 | EF607066 | JF735587 | JF735776 |
| **D. pinicola**    | CBS 139.43           | JF735318 | JF735446 | JF735631 | JF735802 |
| **D. polyphaga**   | CBS 173.37           | JF735319 | JF735447 | JF735614 | JF735803 |
| **D. torresensis** | CBS 119.41           | JF735349 | JF735478 | JF735657 | JF735846 |
| **D. torresensis** | Cyl102               | KP823905 | KP823855 | KP823894 | KP823874 |
| **D. torresensis** | Cyl106               | KP823907 | KP823877 | KP823895 | KP823876 |
| **D. torresensis** | Cyl110               | KP823908 | KP823896 | KP823897 | KP823877 |
| **D. torresensis** | Cyl124               | KP823912 | KP823890 | KP823900 | KP823881 |
| **D. torresensis** | CBS 129086           | JF735362 | JF735492 | JF735681 | JF735769.1 |
| **D. torresensis** | Cyl102               | KP823905 | KP823855 | KP823894 | KP823874 |
| **D. torresensis** | Cyl106               | KP823907 | KP823877 | KP823895 | KP823876 |
| **D. torresensis** | Cyl110               | KP823908 | KP823896 | KP823897 | KP823877 |
| **D. torresensis** | Cyl124               | KP823912 | KP823890 | KP823900 | KP823881 |
| **D. torresensis** | CBS 129082           | JF735303 | JF735431 | JF735580 | JF735769.1 |
| **D. valentina**   | CBS 142826           | KY676881 | KY676875 | KY676863 | KY676869 |
| C. addiensis       | CBS 143794           | MH111383 | MH111388 | N/A      | MH111393 |
| C. addiensis       | CBS 143793           | MH111385 | MH111390 | N/A      | MH111395 |
| C. addiensis       | CBS 143795           | MH111384 | MH111389 | N/A      | MH111394 |
| C. arbusa          | CMW47295/ CBS 143546 | MH017015 | MH016958 | MH016996 | MH016977 |
| C. arbusa          | CMW 47296; CBS 143547| MH017016 | MH016959 | MH016997 | MH016978 |
| C. australiensis   | CBS 129567           | JN100624 | JN098747 | JN098932 | JN099060 |
| C. brevistipitata  | CBS 142786           | N/A      | MF444926 | N/A      | MF444940 |
| C. camelliae       | IMI 346845           | AF220952 | AY793471 | AY793509 | JN099087 |
| C. clavata         | CBS 129564           | JN099095 | JN098752 | JN098858 | JN098974 |
| C. cymbiformis     | CBS 129553           | JN099103 | JN098753 | JN098866 | JN098988 |
| C. elegans         | CBS 338.92           | AY793444 | AY793474 | AY793512 | JN099039 |
| C. ellipsoida      | CBS 129573           | JN099094 | JN098757 | JN098857 | JN098973 |
| C. hahajimaensis   | MAEF238172           | JN687561 | N/A      | JN098761 | JN099057 |
| C. hawaiiensis     | CBS 129569           | JN100621 | JN098761 | JN098929 | JN099057 |
| C. horticola       | CBS 142784           | MF444911 | MF444924 | N/A      | MF444938 |
| C. humicola        | CBS 142779           | MF444906 | MF444919 | N/A      | MF444933 |
| C. infestans       | CBS 111795           | AF220955 | AF220190 | AY793513 | JN099037 |
| C. kurandica       | CBS 129577           | JN100646 | JN098765 | JN098953 | JN099083 |
| C. lageniformis    | CBS 111060           | JN100611 | JN098770 | JN098918 | JN099046 |
| C. lageniformis    | CBS 111061           | JN100606 | JN098771 | JN098913 | JN099040 |
| C. lageniformis    | CBS 112898           | AY793445 | AY725652 | AY725699 | JN098990 |
| C. lageniformis    | CBS 340.92           | MH1862360| AY793481 | AY793520 | JN099003 |
| C. lageniformis    | JZB33200001          | MN988714 | MN958526 | MN958537 | MN958535 |
| C. lageniformis    | JZB33200002          | MN988715 | MN958527 | MN958538 | MN958536 |
| C. lanceolata      | CBS 129566           | JN099099 | JN098789 | JN098862 | JN098978 |
| C. lateralis       | CBS 142788           | MF444914 | MF444928 | N/A      | MF444942 |
| C. longiphialidica | CBS 129557           | JN100585 | JN098790 | JN098851 | JN098966 |
| C. longistipitata  | CBS 116075           | AF220958 | AY793506 | AY793546 | JN098993 |
| C. malesiana       | CBS 143549           | MH017017 | MH016960 | MH016998 | MH016979 |
| C. microcylindrica | CBS 111794           | AY793452 | AY793483 | AY793523 | JN099041 |
| C. natalensis      | CBS 114943           | JN100588 | JN098794 | JN098895 | JN099016 |

(Continued)
old healthy plants of grapevine cv. ‘Summer Black’. Five isolates (JZB3320001, JZB3310007, JZB3310008, JZB33100011 and JZB3210004) were selected randomly for pathogenicity tests. Mycelium discs (4 mm diam.) were obtained from the edges of PDA colonies which were grown for 10 d at 25°C.

Detached shoots were surface-disinfected in 75% ethanol and then dried, and each shoot was then wounded (4 mm) using a sterilized scalpel. The mycelium discs were placed onto the wound sites and covered with parafilm (Bemis). Non-colonized sterile PDA plugs were used as negative controls. The shoots were then inserted into moist soil and kept at 25°C. Each experiment included ten shoots for each fungus isolate, with a total of three parallel experiments conducted. The lengths of the lesions were measured after 7 d, and meanwhile photos were taken.

Pathogenicity tests of BF fungal agents were further conducted on the 3-month old grapevine cuttings which were inoculated in a manner similar to the detached green shoots. The experiment was performed in six cuttings for each tested isolate and the negative controls. The plants were kept in a greenhouse maintained at 25°C, and the trial was conducted twice. Shoots were collected, and lesion lengths were measured upward and downward from the points of inoculation after 80 d.

Fungi were re-isolated from necroses on the test plants in all pathogenicity tests, and fungus identifications were based on cultural and morphological characters. The lesion dimension data were statistically ana-
compared to those deposited in GenBank, and the iso-
regions were approx. 0.5 kb. All sequences obtained were
amplified for all the isolates. The products of the ITS
genus confirmation of the isolates, the ITS regions were
obtained from the symptomatic grapevine tissues. For
after 14 d of growth on PDA. In total, 50 isolates were
vineyards was 0.1% to 1%. Fungus isolation and initial species identifications

RESULTS

Incidence of BF-like symptoms in the investigated
vinyards was 0.1% to 1%. Colony morphology of all the isolates distinguished
after 14 d of growth on PDA. In total, 50 isolates were
obtained from the symptomatic grapevine tissues. For
genus confirmation of the isolates, the ITS regions were
amplified for all the isolates. The products of the ITS
regions were approx. 0.5 kb. All sequences obtained were
compared to those deposited in GenBank, and the iso-
lates possessed 95%-99% similarity with sequences from
the genera *Cylindrocladiella*, *Dactylonectria* or *Neo-
ectria*. One or two isolates were selected from each of these
tree genera for pathogenicity tests.

The ML MP trees had similar topologies, so only
the ML tree is presented in this study, with ML and MP
bootstrap support values.

Identification of Cylindrocladiella species The optimization likelihood value of the final ML tree
was -12848.031218. The matrix had 916 distinct alignment patterns, with 16.70% of undetermined characters or gaps. Parameters for the GAMMA+P-Invar model were: estimated base frequencies $A = 0.216108$, $C = 0.328348$, $G = 0.225791$, $T = 0.229753$; substitution rates include $TL = 1.593283$, $AC = 1.370476$, $AG = 3.210224$, $AT = 1.626079$, $CG = 0.702088$, $CT = 5.644898$, $GT = 1.000000$; proportion of invariable sites (I) = 0.388010, and gamma distribution
shape parameter ($\alpha$) = 0.971160. In the MP tree, the
heuristic search produced 1000 trees (length = 1981, CI
= 0.610, RI = 0.911, RC = 0.556 and HI = 0.390), and the
dataset consisted of 2041 total characters. Of these, 1225
were constant, 88 variable characters were parsimony
informative and 728 were parsimony-informative. In
the ML tree (Figure 3), generated using the combined data,
three isolates (IZB3310008, IZB3310009 and IZB3310010)
collected from Ningxia province clustered with *D. macro-
didyma* (CBS 112615) and one isolate (IZB3210004) clus-
tered with *Neonectria* sp. 1(CPC 13545); In addition, the
isolate (IZB3310007) clustered with *D. alacerensis* (CBS
129087) and two isolates (IZB33100011 and IZB33100012)
clustered with *D. torresensis*(CBS 119.41).

Morphological characteristics

Morphological observations for the five identified
species are outlined below.

*Cylindrocladiella lageniformis* Crous, M.J. Wingf. & Alfe-
nas
Pathogenic on trunks and rootstocks of *Vitis vinifera*. Asexual morph: Conidiophores were hyaline and pen-
icillate. Conidia were hyaline, cylindrical, one septate or
aspelet, with dimensions of 5.3-9.5 × 1.5-2.8 µm, mean ± SD = 7.8 ± 1.1 × 2.1 ± 0.3 µm. The terminal vesicles were lageniform to ovoid. (Figure 4 c-d). Sexual morph: undetermined.

Culture characteristics: Colonies on PDA reached 74.9 ±
0.8 mm diam. after 6 d incubation at 25°C in the dark,
and were yellow to tan, with flourish aerial mycelium
(figure 4 a-b).

Material examined: CHINA, Guangxi province, on
trunk and rootstock of *Vitis vinifera*, 8 April 2018, Xing-
hong Li, living cultures, IZB3320001, IZB3320002.

*Dactylonectria macrodidyma* (Halleen, Schroers &
Crous) L. Lombard & Crous
Pathogenic on trunks and roots of *Vitis vinifera*. Asex-
ual morph: the isolates rarely formed chlamydospores
and microconidia, producing abundant macroconidia
on MEA. Macroconidia hyaline, cylindrical, straight
to slightly curved, one to four septate, with dimensions
Figure 2. Maximum likelihood tree obtained from the phylogenetic analysis based on tef1, his3 and tub2 sequence alignments. The scale bar represents 0.07 changes. The tree is rooted in Gliocladiopsis sagariensis (CBS 199.55).
Figure 3. Maximum likelihood tree obtained from the phylogenetic analysis based on combined ITS, tub2, his3, and tef1 sequence alignments. The scale bar represents 0.05 changes. The ex-type strains are in bold font. The outgroups of the tree are Campylocarpon fasciculare (CBS 112613) and C. pseudofasciculare (CBS 112679).
Fungal pathogens associated with black foot of grapevine in China

of 14.4–44.2 × 4.0–8.2 μm, mean ± SD = 31.1 ± 7.8×6.2 ± 0.9 μm (Figure 4, g and h). Microconidia ellipsoid to ovoid, hyaline, straight, aseptate to one septate. **Sexual morph**: undetermined.

**Culture characteristics**: Colonies on PDA reached 57.3 ± 5.4 mm diameter after 9 d at 25°C in the dark, and were yellowish, with abundant aerial mycelium (Figure 4e). Colony reverse sides were burnt umber to raw sienna or brownish yellow on PDA (Figure 4f).

**Material examined**: CHINA, Ningxia province, on trunk and rootstock of *Vitis vinifera*, 8 April 2018, Qingtong Ye and Xinghong Li, living cultures, JZB3310008, JZB3310009, JZB3310010.

*Dactylonectria torresensis* (A. Cabral, Rego & Crous) L.
Pathogenic on trunks and rootstocks of *Vitis vinifera*. **Asexual morph:** The isolates rarely formed chlamydospores and microconidia, producing some macroconidia on MEA. Macroconidia straight or minutely curved, cylindrical, one to four septate. Microconidia zero to one septate, ellipsoid to ovoid. **Sexual morph:** undetermined. **Culture characteristics:** Colonies on PDA reached 55.5 ± 3.6 mm diam. after 9 d at 25°C in the dark, and were pale buff to chestnut (Figure 4, i and j). Colony reverse sides were buff to umber to chestnut on PDA. **Material examined:** CHINA, Shanxi and Hebei province, on trunk and rootstock of *Vitis vinifera*, 8 April 2018, Qingtong Ye and Xinghong Li, living cultures, JZB3310011, JZB3310012.

*Dactylonectria alcacerensis* (A. Cabral, H. Oliveira & Crous) L. Lombard & Crous

Pathogenic on roots of *Vitis vinifera*. **Asexual morph:** isolates did not produce macroconidia, microconidia, or chlamydospores on MEA. **Sexual morph:** undetermined. **Culture characteristics:** Colonies on PDA reached 49.3 ± 2.2 mm diam. after 9 d at 25°C in the dark, and were feltly to slightly cottony (Figure 4 m-n). **Material examined:** CHINA, Shanxi province, on trunk and rootstock of *Vitis vinifera*, 8 May 2018, Qingtong Ye and Xinghong Li, living culture, JZB3310007.

*Neonectria* sp. 1

Pathogenic on the bark of trunk of *Vitis vinifera*. **Asexual morph:** In the present study, the isolates did not produce macroconidia, microconidia, or chlamydospores on PDA. **Sexual morph:** undetermined. **Culture characteristics:** Colonies on PDA reached 61.1±1.1 mm diameter after 15 d of incubation at 20°C in dark (Figure 4 o-p). **Material examined:** CHINA, Xinjiang province, on trunk and rootstock of *Vitis vinifera*, 16 April 2018, Qingtong Ye and Xinghong Li, living culture,

![Figure 5](image-url)
Pathogenicity tests

In the pathogenicity tests conducted with detached green shoots, the non-inoculated shoots did not develop any symptoms (Figure 5a). In contrast, shoots inoculated with mycelium discs resulted in necroses. The lesions were brown to black, and the mean lesion lengths differed among the different inoculated fungi ($P < 0.05$) (Figure 5, b to h). *Dactylonectria macrodidyma* was the most aggressive pathogen (mean lesion length = 1.18 cm) among the five species (Figure 5). The re-isolation rates of isolates *C. lageniformis*, *D. torresensis*, *D. macrodidyma*, *D. alcacerensis*, and *Neonectria* sp. 1. were between 70% with 100% from the lesions.

Pathogenicity tests on 3-month-old grapevine cuttings showed different results for the different inoculated pathogens, as well. The non-inoculated controls showed no symptoms on the shoots (Figure 6a). *Dactylonectria macrodidyma* caused brown to black necrotic lesions on the shoots (mean lesion length = 1.95 cm) (Figure 6, d and e). Less necrosis was observed in the cuttings inoculated in *C. lageniformis*, *D. torresensis*, *Neonectria* sp. 1., or *D. alcacerensis* (Figure 6, b to c', f to g'). The re-isolation rates of the different inoculated fungi from the respective lesions were between 70% with 100%.

This is the first report of *C. lageniformis*, *D. torresensis*, *D. macrodidyma*, *D. alcacerensis* and *Neonectria* sp. 1 associated with BF of grapevines in China.

DISCUSSION

Grapevines can be affected by several diseases throughout each year, especially during fruit production. In the present study, 50 isolates obtained from diseased grapevine samples in five provinces of China were identified as *C. lageniformis*, *D. torresensis*, *D. macrodidyma*, *D. alcacerensis*, or *Neonectria* sp. 1. To date, *D. torresen-

![Figure 6](image-url)

*Figure 6.* Pathogenicity tests results (after 80 d) of BF fungal agents inoculated onto 3-month-old ‘Summer Black’ grapevine plants in greenhouse. Control (a), *Cylindrocladiella lageniformis* (b and b’), *Dactinonectria torresensis* (c and c’), *Dactylonectria macrodidyma* (d and e), *D. alcacerensis* (f and g), *Neonectria* sp. 1 (g’). Histogram (h) of mean lesion lengths caused by inoculations with the different fungi. Means accompanied by different letters are significantly different ($P = 0.05$).
Berlanas also reported in California and Spain (Koike et al., 2016; et al., most of the fungi reported in the present study were been conducted with green shoots (Van Coller et al., 2010; Halleen and Fourie 2016), fungicides (Halleen et al., 2007; Rego et al., 2009; Alaniz et al., 2011) and biological control agents (Berbegal et al., 2020; Martínez-Diz et al., 2021; van Jaarsveld et al., 2020, 2021). Chemical treatments during propagation processes in nurseries for control of BF pathogens have been evaluated, including treating cutting prior to cold storage, cutting prior to callusing, rooting pre- and post-grafting, and pre-planting fungicide treatments of rooted cuttings, to eliminate or reduce potential fungal agents before planting (Halleen et al., 2007; Rego et al., 2009, Alaniz et al., 2011, Gramaje et al., 2018). Based on previous research, benomyl was effective for elimination or reducing Cylindrocarpon destructans infections (Rego et al. 2006). Reductions of D. torresensis and D. macrodidyma incidence and disease severity on the bases of 2-year-old plants have been reported from applications of Streptomyces sp. E1+R4 before preplanting (Martínez-Diz et al., 2021).

Although the incidence of diseased plants with BF symptoms was about 1% in the surveyed vineyards in China, which is much less than in France (losses of 50%: Larignon et al., 1999), BF pathogens can infect grapevine roots and trunks in young nurseries and plantations, and the pathogenic fungi can be transmitted to new vineyards by cuttings (De la Fuente et al., 2016). The fungi C. lageniformis, D. torresensis, D. macrodidyma, D. alcacerensis, and Neonectria sp. 1. are all soilborne, and can infect hosts through the soil (Halleen et al., 2003). In the north of China, grapevines need to be buried under the soil for survival during cold weather, resulting in small wounds that are likely to be susceptible to infection by soilborne fungi, so more attention should be paid to BF in China in future.

Grapevine BF is prevalent in nurseries and new plantations (De la Fuente et al., 2016), and the current strategies for controlling this disease include good hygiene or sanitation, which are the most important means of obtaining healthy vines (Gramaje and Armengol 2011), including treatments with hot water, (Gramaje et al., 2010; Halleen and Fourie 2016), fungicides (Halleen et al., 2007; Rego et al., 2009; Alaniz et al., 2011) and biological control agents (Berbegal et al., 2020; Martínez-Diz et al., 2021; van Jaarsveld et al., 2020, 2021). Chemical treatments during propagation processes in nurseries for control of BF pathogens have been evaluated, including treating cutting prior to cold storage, cutting prior to callusing, rooting pre- and post-grafting, and pre-planting fungicide treatments of rooted cuttings, to eliminate or reduce potential fungal agents before planting (Halleen et al., 2007; Rego et al., 2009, Alaniz et al., 2011, Gramaje et al., 2018). Based on previous research, benomyl was effective for elimination or reducing Cylindrocarpon destructans infections (Rego et al. 2006). Reductions of D. torresensis and D. macrodidyma incidence and disease severity on the bases of 2-year-old plants have been reported from applications of Streptomyces sp. E1+R4 before preplanting (Martínez-Diz et al., 2021).

Some practices, such as hot water treatments, are useful for sanitizing commercially produced plants. Generally, this practice entails treating the plants at 50°C for 30 min. However, this is stressful for the plants (Waite et al., 2013). Despite treated with these practices, diseases in symptomless plants can still be transmitted to non-infested areas (De la Fuente et al., 2016). The detection of BF fungi in soils or vines is essential for controlling the disease in nurseries and new plantations. Alaniz et al. (2009a) reported a multiplex PCR system for specific and early detection of Ilyonectria liriodendri (=Cylindrocarpon liriodendron), Dactylonectria macro-
ACKNOWLEDGEMENTS

The project was funded by 2018YFD0201301, CARS-29 and JKZX201905. The authors thank the Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Sciences for providing 1-year-old ‘Summer black’ dormant shoots, and grapevine farmers for their co-operation.

LITERATURE CITED

Abreo E., Martínez S., Betucci L., Lupo S., 2010. Morphological and molecular characterization of Campylocarpon and Cylindrocarpon spp. associated with black foot disease of grapevines in Uruguay. Australasian Plant Pathology 39: 446–452.

Agustí-Brisach C., Armengol J., 2013. Black-foot disease of grapevine: an update on taxonomy, epidemiology and management strategies. Phytopathologia Mediterranea 52: 245–261.

Agustí-Brisach C., Gramaje D., García-Jiménez J., Armengol J., 2013. Detection of black-foot and Petri disease pathogens in natural soils of grapevine nurseries and vineyards using bait plants. Plant and Soil 364: 5-13.

Agustí-Brisach C., Mostert L., Armengol J., 2014. Detection and quantification of Ilyonectria spp. associated with black-foot disease of grapevine in nursery soils using multiplex nested PCR and quantitative PCR. Plant Pathology 63: 316–322.

Aigoun-Mouhous W., Elena G., Cabral A., León M., Sabaou N., … Berraf-Tebbal A., 2019. Characterization and pathogenicity of Cylindrocarpon-like asexual morphs associated with black foot disease in Algerian grapevine nurseries, with the description of Pleio-

carpon algeriense sp. nov. European Journal of Plant Pathology 154: 887–901.

Alaniz S., Leon M., García-Jiménez J., Abad P., Armengol, J., 2007. Characterization of Cylindrocarpon species associated with black-foot disease of grapevine in Spain. Plant Disease 91: 1187–1193.

Alaniz S., Armengol J., García-Jiménez J., Abad-Campos P., León M., 2009a. A multiplex pcr system for the specific detection of Cylindrocarpon liriodendri, C. macrodidymum, and C. pauciseptatum from grapevine. Plant Disease 93: 821–825.

Alaniz S., Armengol J., León M., García-Gimenez J., Abad-Campos P., 2009b. Analysis of genetic and virulence diversity of Cylindrocarpon liriodendri and C. macrodidymum associated with black foot disease of grapevine. Mythological Research 113:16–23.

Alaniz S., Abad-Campos P., García-Jiménez J., Armengol J., 2011. Evaluation of fungicides to control Cylindrocarpon liriodendri and Cylindrocarpon macrodidymum in vitro, and their effect during the rooting phase in the grapevine propagation process. Crop Protection 30: 489–494.

Berbegal M., Ramón-Albalat A., León M., Armengol J., 2020. Evaluation of long-term protection from nursery to vineyard provided by trichoderma atroviride scl1 against fungal grapevine trunk pathogens. Pest Management Science 76: 967–977.

Berlanas C., Ojeda S., López-Manzanares B., Andrés-Sodupe M., Bujanda R., … Gramaje D., 2020. Occurrence and diversity of black-foot disease fungi in symptomless grapevine nursery stock in Spain. Plant Disease 104: 94–104.

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

Carlucci A., Lops F., Mostert L., Halleen F., Raimondo M. L., 2017. Occurrence fungi causing black foot on young grapevines and nursery rootstock plants in Italy. Phytopathologia Mediterranea 56: 10–39.

Crous P. W., Groenewald J. Z., Risede J. M., Simoneau P., Hywel-Jones N. L., 2004. Calonectria species and their Cylindrocladium anamorphs: species with sphaeroapedunculate vesicles. Study in Mycology 50: 415–430.

Dissanayake A. J., Mei L., Wei Z., Zhen C., Udayanga D., … Hyde K. D., 2015. Morphological and molecular characterisation of Diaporthe species associated with grapevine trunk disease in China. Fungal Biology 119: 283–294.

De la Fuente M., Fontaine F., Gramaje D., Armengol J., … Corio-Costet M.F., 2016. Grapevine Trunk Diseases A Review. OIV Publications, 1st Edn. Paris. https://

Fungal pathogens associated with black foot of grapevine in China
www.oiv.int/public/medias/4650/trunk-diseases-oiv-2016.pdf

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

Gramaje D., Alaniz S., Abad-Campos P., Garcia-Jimenez J., Armengol J., 2010. Effect of hot-water treatments in vitro on conidial germination and mycelial growth of grapevine trunk pathogens. *Annals of Applied Biology* 156: 231–241.

Gramaje D., Armengol J., 2011. Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. *Plant Disease* 95: 1040–1055.

Gramaje D., Úrbez-Torres J. R., Sosnowski M. R., 2018. *Ampelography Society* 89–90.

Halleen F., Huisman J., Halleen F., Fourie P. H., Crous P. W., 2016. An Integrated Strategy for the Proactive Management of Grapevine Trunk Disease Pathogen Infections in Grapevine Nurseries. *South African Journal for Enology and Viticulture* 37: 104–114.

Hofstetter V., Buyck B., Croll D., Viret O., Couloux A., Gindro K., 2012. What if esca disease of grapevine were not a fungal disease? *Fungal Diversity* 54: 51–67.

Katoh K., Rozewicki J., Yamada K. D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166.

Kishino H., Hasegawa M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* 29: 170–179.

Koike S.T., Bettiga L.J., Nguyen T.T., and Gubler W.D., 2016. First report of *Cylindrocladiella lageniformis* and *C. peruviana* as grapevine pathogens in California. *Plant Disease* 100: 1783–1784.

Kuraku S., Zmasek C. M., Nishimura O., Katoh K. 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Research* 41: W22-W28.

Langenhoven S. D., Halleen F., Spies C. F. J., Stempien E., Mostert L., 2018. Detection and quantification of black foot and crown and root rot pathogens in grapevine nursery soils in the Western Cape of South Africa. *Phytopathologia Mediterranea* 57: 519–537.

Larignon P., 1999. Black foot disease in France. In: (Morton L., ed.) *Proceedings of the Seminar and Workshop on Black Goo Symptoms and Occurrence of Grape Declines*. Fort Valley, VA, USA: International Ampelography Society 89–90.

Lawrence D. P., Nouri M. T., Trouillas F. P., 2019. Taxonomy and multi-locus phylogeny of *Cylindrocarpon*-like species associated with diseases roots of grapevine and other fruit and nut crops in California. *Fungal Systematics and Evolution* 4: 59–75.

Li H., Li R. Y., Wang H. 2007. New Disease for Winemaking Grape-Eutypa Dieback[J]. *Liquor-Making Science & Technology* 155: 48–50.(in Chinese)

Li X. H., Yan J. Y., Kong F. F., Qiao G. H., Zhang Z. W., Wang Z. 2010. *Botryosphaeria dothidea* causing canker of grapevine newly reported in China. *Plant Pathology* 59: 1170–1170.

Lombard L., Van Der Merwe N. A., Groenewald J. Z., Crous P. W., 2014. Lineages in *Nectriaceae*: re-evaluating the generic status of *Nectria* and allied genera. *Phytopathologia Mediterranea* 53: 515–532.

Manawasinghe I. S., Dissanayake A. J., Xing H.L., Mei L., Wanasinghe D. N., … Ji Y.Y., 2019. High genetic diversity and species complexity of *Diaporthe* associated with grapevine dieback in China. *Frontiers in Microbiology* 10: 1936.

Marin-Felix Y., Hernández-Restrepo M., Wingfield M. J., Akulov A., Carnegie A. J., … Crous P.W., 2019. Genera of phytopathogenic fungi: GOPHY 2. *Studies in Mycology* 92: 47–133.
Fungal pathogens associated with black foot of grapevine in China

Martínez-Diz M. P., Andrés-Sodupe M., Berbegal M., Bujanda R., Gramaje D., 2020. Droplet digital PCR technology for detection of Ilyonectria liriodendri from grapevine environmental samples. Plant Disease 104: 1144–1150.

Martínez-Diz M.P., Diaz-Losada E., Andrés-Sodupe M., Bujanda R., Maldonado-González M.M., ... Gramaje D., 2021. Field evaluation of biocontrol agents against black-foot and Petri diseases of grapevine. Pest Management Science 77: 697–708.

O’Donnell K., Cigelnik E., 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Molecular Phylogenetics and Evolution 7: 103–116.

Pecenka J., Eichmeier A., Penazova E., Baranek M., Leon M., Armengol J. 2018. First report of Dactylonectria torresensis causing black-foot disease on grapevines in the Czech Republic. Plant Disease 102: 2038–2039.

Petit E., Barriault E., Baumgartner K., Wilcox W. F., Rolshausen P. E., 2011. Cylindrocarpon species associated with black-foot of grapevine in northeastern United States and Southeastern Canada. American Journal of Enology and Viticulture 62: 177–183.

Pintos C., Redondo V., Costas D., Aguin O., Mansilla P., 2019. Fungi associated with grapevine trunk diseases in nursery-produced Vitis vinifera plants. Phytopathologia Mediterranea 57: 407–424.

Probst C. M., Ridgway H. J., Jaspers M. V., Jones E. E., 2019. Pathogenicity of Ilyonectria liriodendri and Dactylonectria macrodidyma propagules in grapevines. European Journal of Plant Pathology 154: 405–421.

Rambaut A., FigTree v1.4.4: Tree figure drawing tool. 2018. https://github.com/rambaut/figtree/releases

Rego C., Oliveira H., Carvalho A., Phillips A., 2000. Involvement of Phaeoacremonium spp. and Cylindrocarpon destructans with grapevine decline in Portugal. Phytopathologia Mediterranea 39: 76–79.

Rego C., Farropas L., Nascimento T., Cabral A., Oliveira H. 2006. Black foot of grapevine, sensitivity of Cylindrocarpon destructans to fungicides. Phytopathologia Mediterranea 45: 93–100.

Rego C., Nascimento T., Cabral A., Silva M. J., Oliveira H., 2009. Control of grapevine wood fungi in commercial nurseries. Phytopathologia Mediterranea 48: 128–135.

Silvestro D., Michalak I., 2016. RaxmlGUI: a graphical front-end for RAxML. Retrieved at 29 December 2016, from http://sourceforge.net/projects/raxmlgui/.

Swofford D. L., 2002. PAUP* 4.0: phylogenetic analysis using parsimony (and other methods). Sinauer Associates, Sunderland, Massachusetts, USA.

Udayanga D., Liu, X. Z., Croux, P. W., McKenzie, E. H. C., Chukeatirote, E., and Hyde, K. D., 2012a. A multi-locus phylogenetic evaluation of Diaporthe (Phomopsis). Fungal Diversity 56: 157–171.

Udayanga D., Liu X. X., Crous P. W., McKenzie E. H. C., Chukeatirote E., Hyde K. D., 2012b. Multilocus phylogeny of Diaporthe reveals three new cryptic species from Thailand. Cryptogamie Mycologie 33: 295–309.

Van Coller G.J., Denman S., Groenewald J.Z., Lamprecht S.C., and Crous P.W., 2005. Characterisation and pathogenicity of Cylindrocladiella spp. associated with root and cutting rot symptoms of grapevines in nurseries. Australasian Plant Pathology 34: 489–498.

van Jaarsveld W. J., Halleen F., Mostert L., 2020. In vitro screening of Trichoderma isolates for biocontrol of black foot disease pathogens. Phytopathologia Mediterranea 59: 465–471.

van Jaarsveld, W. J., Halleen F., Bester M. C., Pierron R. J., Stempien E., Mostert L., 2021. Investigation of Trichoderma species colonization of nursery grapevines for improved management of black foot disease. Pest Management Science 77: 397–405.

Waite H., May P., Bossinger G., 2013. Variations in phytosanitary and other management practices in Australian grapevine nurseries. Phytopathologia Mediterranea 52: 369–379.

White T. J., Bruns T., Lee S., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. M.A. Innis, D.H. Gelband, J.J. Sninsky and T.J. White, eds. Academic Press Inc., New York, NY, USA, 315–322.

Wilcox W. F., Gubler W. D., Uyemoto J. K., 2006. Compendium of Grape Diseases, Disorders, and Pests, Second Edition. American Phytopathological Society, St. Paul, Minnesota, USA, 232 pp.

Yan J. Y., Yue X., Wei Z., Yong W., … Xing H.L., 2013. Species of Botryosphaeriaceae involved in grapevine dieback in China. Fungal Diversity 61: 221–236.

Ye Q.T., Manawasinghe I.S., Wei Z., Mugnai L., Hyde K.D., Xing H.L., Ji Y.Y., 2020. First Report of Phaeoacremonium minimum associated with grapevine trunk diseases in China. Plant Disease 104: 1259–1259.