Stem Blight of Blueberry Caused by *Lasiodiplodia vaccinii* sp. nov. in China

Lin Zhao,¹ Yu Wang,¹ Wei He,² and Ying Zhang^{1,†}

¹ Institute of Microbiology, Beijing Forestry University, Beijing 100083, People's Republic of China

² Beijing Key Laboratory for Forest Pest Control, Beijing Forestry University, Beijing 100083, People's Republic of China

Abstract

Blueberries (*Vaccinium* spp.) are largely cultivated in China because of their nutritional benefits and economic value. Blueberry stem blight caused by members of the Botryosphaeriaceae has become one of the most severe diseases affecting blueberry cultivation in China. In this study, we examined the causal agent of blueberry stem blight at commercial greenhouse farms in the suburban area of Beijing, China. In total, 37 isolates of Botryosphaeriaceae were obtained from 100 stem blight samples of blueberry. Twelve of 37 strains were morphologically consistent with the genus *Lasiodiplodia*, showing ellipsoid to ovoid, one-celled, hyaline conidia that sometimes turned brown, with median septa and longitudinal striations when mature. These 12 strains were identified as belonging to a novel fungal species, *Lasiodiplodia vaccinii*, based on phylogenetic analysis of the concatenated internal transcribed spacer, RNA polymerase II gene, β -tubulin gene,

and translation elongation factor-1 α gene sequences as well as morphological characteristics. Pathogenicity tests indicated that *L. vaccinii* can cause twig blight on blueberry seedlings in the greenhouse. Mycelial growth of *L. vaccinii* occurred at pH values ranging from 3.0 to 10.0, with an optimum at 6.2, and at temperatures from 15 to 40°C, with an optimum at 30.3°C. Of the seven carbon sources tested, sucrose, fructose, and glucose were all highly efficient in supporting the mycelial growth of *L. vaccinii*, and xylose was the least effective. Of six nitrogen sources tested, yeast extract and tryptone best promoted mycelial growth of *L. vaccinii*. The ability of *L. vaccinii* to grow at high temperatures may help to explain its occurrence in Beijing greenhouses in this study.

Keywords: fruit, fungi, small fruits, tree fruits, pathogen diversity

Blueberries (*Vaccinium* spp.), one of the most nutritious and antioxidant-rich fruits, can enhance memory and prevent heart disease (Shi and Liu 2009). Because of their health benefits and economic value, blueberries are commercially cultivated worldwide, including in the United States, Canada, and Europe (Evans and Ballen 2014; Popović et al. 2018). In China, blueberry cultivation started in 1981, and there has been a significant increase in the area under cultivation, with >31,210 ha and productivity of 43,244 tons per year over the last 10 years (Li et al. 2016).

The Botryosphaeriaceae family is composed of versatile and cosmopolitan species associated with a variety of plants, including *Vaccinium* spp., as pathogens, endophytes, or saprophytes (Phillips et al. 2013; Slippers and Wingfield 2007). Members of the Botryosphaeriaceae have been reported as causal agents of cankers, dieback, fruit blights, and seed rots of woody plants at many locations across the world (Chen et al. 2015; Milholland 1972a, b; Phillips et al. 2006; Tang et al. 2012; van Niekerk et al. 2004). For instance, shoot blight of the genus *Pistacia* caused by botryosphaeriaceous fungi is regarded as a major threat to the pistachio industry in the United States and Greece (Chen et al. 2014, 2015; Michailides and Morgan 1992, 2004). *Botryosphaeria dothidea* is the causal agent of Botryosphaeria canker of apple and apple ring rot, which is one of the most

severe diseases affecting apple production in China (Tang et al. 2012).

The botryosphaeriaceous fungi have been widely regarded as the main causal agents of blueberry stem blight (Boyzo-Marin et al. 2016; Creswell and Milholland 1988; Espinoza et al. 2009; Koike et al. 2014; Milholland 1972 a, b; Popović et al. 2018; Smith 2004; Tennakoon et al. 2018; Witcher and Clayton 1962; Wright and Harmon 2009, 2010; Xu 2016; Xu et al. 2015; Yu et al. 2012, 2013a, b). Symptoms associated with blueberry stem blight include wilting of the twigs and infected woody tissue turning brown, and pathogens invade the xylem, resulting in mortality of whole plants (Milholland 1972b; Wright and Harmon 2010; Xu 2016; Xu et al. 2015). Several members of the Botryosphaeriaceae have been reported as causing stem blight or dieback of blueberries in China, including *B. dothidea*, *Neofusicoccum parvum*, *Neofusicoccum vitifusiforme*, *Lasiodiplodia theobromae*, *Lasiodiplodia pseudotheobromae*, and *Lasiodiplodia chinensis* (Dou et al. 2017b; Kong et al. 2010; Wang et al. 2016; Xu 2016; Xu et al. 2015; Yu et al. 2012, 2013a, b).

Stem blight and branch blight have occurred on highbush blueberries (*Vaccinium corymbosum* L.) at commercial greenhouse farms in the suburbs of Beijing, China. Typical symptoms of the disease are stem blight and dieback with lesions extending along entire branches. Infected stems have a light brown to brown pigmentation. The dead leaves stay on the recently killed stems as a brown “flag.” The disease seriously affects the growth of blueberry plants, fruit quality, and productivity, with crop damage ranging from 15 to 25% based on our observations. The objectives of this study were to (i) identify the pathogenic fungi causing blueberry stem blight disease on the basis of morphological characteristics and multigene sequence analysis, (ii) determine their pathogenicity on blueberry, and (iii) characterize their growth at different temperatures and pH and their capacity to utilize different carbon and nitrogen sources.

Materials and Methods

Sample collection and fungal isolation. Samples were collected in the greenhouse at Huiyuan Juice Blueberry Production Garden in the suburb area of Beijing, China. Diseased or dead stems (about 30 cm in length) were taken from blueberry branches

[†]Corresponding author: Y. Zhang; yzhang@bjfu.edu.cn

L. Zhao and Y. Wang contributed equally to this work.

Funding: This work was supported by Fundamental Research Funds for the Central Universities grant 2016ZCQ04; National Natural Science Foundation of China (NSFC) General Program and grants 31770015 and 31370063; NSFC Projects of International Cooperation and Exchanges grant 3155461143028; and National Science and Technology Foundation Project grant 2014FY210400.

The author(s) declare no conflict of interest.

Accepted for publication 18 March 2019.

(*V. corymbosum*) in October 2017. In total, 100 samples were collected from 50 bushes (with one dead twig and one diseased twig for each bush). Wood segments (0.5 × 0.5 × 0.2 cm) cut from the disease lesion boundary or dead tissue were surface disinfected with 75% ethanol for 30 s and rinsed three times with sterile water; then, they were incubated on malt extract agar (MEA; 2%) for isolation of fungal strains (Pavlic et al. 2004; Xu et al. 2015). Petri dishes were incubated in the dark at 28°C until the fungal colonies were observed. Colonies resembling the Botryosphaeriaceae were obtained by transferring hyphal tips from colony margins to MEA plates. Isolates grown on MEA in the dark were kept at 28°C to determine colony characteristics.

Morphological characterization. Fungal colonies were initially identified based on morphological characteristics, including colony,

conidiogenous cells, and conidia. To induce sporulation, isolates were cultivated on water agar (WA; 2%; Biolab, S.A.) with autoclaved pine needles placed on the medium and incubated at 25°C under near-ultraviolet light (mainly 340 nm) (Dou et al. 2017a). Pycnidia produced on the pine needles were morphologically described and characterized following the protocol of Dou et al. (2017a, b). Measurements of 20 paraphyses, 20 conidiogenous cells, and 50 conidia were made by immersing pycnidia in water under a microscope (Nikon Eclipse E600). Fungal isolates and specimens were deposited at Beijing Forestry University, with duplicates at the China General Microbiological Culture Collection Center (CGMCC) and the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS) (Table 1).

Table 1. GenBank accession numbers of isolates included in this study^y

Species	Isolate	GenBank accession number ^z			
		ITS	<i>tef1-α</i>	<i>TUB</i>	<i>RPB2</i>
<i>Botryosphaeria dothidea</i>	CBS 115476	AY236949	AY236898	N/A	N/A
<i>Botryosphaeria dothidea</i>	CGMCC 3.19257	MK085913	MK085916	N/A	N/A
<i>Botryosphaeria dothidea</i>	CGMCC 3.19258	MK085912	MK085915	N/A	N/A
<i>Botryosphaeria dothidea</i>	CGMCC 3.19259	MK085911	MK085914	N/A	N/A
<i>Diplodia mutila</i>	CMW 7060	AY236955	AY236904	AY236933	EU339574
<i>Diplodia seriata</i>	CBS 112555	AY259094	AY573220	DQ458856	N/A
<i>Lasiodiplodia americana</i>	CERC 1961	KP217059	KP217067	KP217075	N/A
<i>Lasiodiplodia americana</i>	CERC 1960	KP217058	KP217066	KP217074	N/A
<i>Lasiodiplodia avicenniae</i>	CMW 41467	KP860835	KP860680	KP860758	KU587878
<i>Lasiodiplodia avicenniae</i>	LAS 199	KU587957	KU587947	KU587868	KU587880
<i>Lasiodiplodia brasiliense</i>	CMM 2321	KC484797	KC481528	N/A	N/A
<i>Lasiodiplodia brasiliense</i>	CGMCC 3.17966	KY783475	KY848612	KY848556	KY848595
<i>Lasiodiplodia brasiliense</i>	CMM 4015	JX464063	JX464049	N/A	N/A
<i>Lasiodiplodia brasiliense</i>	CMW 35884	KU887094	KU886972	KU887466	KU696345
<i>Lasiodiplodia bruguierae</i>	CMW 41470	KP860833	KP860678	KP860756	KU587875
<i>Lasiodiplodia bruguierae</i>	CMW 42480	KP860832	KP860677	KP860755	KU587876
<i>Lasiodiplodia caatinguensis</i>	CMM 1325	KT154760	KT008006	KT154767	N/A
<i>Lasiodiplodia caatinguensis</i>	IBL 381	KT154757	KT154751	KT154764	N/A
<i>Lasiodiplodia chinensis</i>	CGMCC 3.18061	KX499889	KX499927	KX500002	KX499965
<i>Lasiodiplodia chinensis</i>	CGMCC 3.18063	KX499891	KX499929	KX500004	KX499967
<i>Lasiodiplodia citricola</i>	IRAN 1522C	GU945354	GU945340	KU887505	KU696351
<i>Lasiodiplodia citricola</i>	IRAN 1521C	GU945353	GU945339	KP872406	KP872456
<i>Lasiodiplodia crassisporea</i>	WAC12533	DQ103550	DQ103557	KU887506	KU696353
<i>Lasiodiplodia crassisporea</i>	CMW 13488	DQ103552	DQ103559	KU887507	KU696352
<i>Lasiodiplodia egyptiaca</i>	BOT 29	JN814401	JN814428	N/A	N/A
<i>Lasiodiplodia euphorbicola</i>	CMW 33350	KU887149	KU887026	KU887455	KU696346
<i>Lasiodiplodia euphorbicola</i>	CMW 36231	KU887187	KU887063	KU887494	KU696347
<i>Lasiodiplodia euphorbicola</i>	CMM 3609	KF234543	KF226689	KF254926	N/A
<i>Lasiodiplodia exigua</i>	CBS 137785	KJ638317	KJ638336	KU887509	KU696355
<i>Lasiodiplodia exigua</i>	BL 184	KJ638318	KJ638337	N/A	N/A
<i>Lasiodiplodia gilanensis</i>	IRAN 1523C	GU945351	GU945342	KU887511	KU696357
<i>Lasiodiplodia gilanensis</i>	IRAN 1501C	GU945352	GU945341	KU887510	KU696356
<i>Lasiodiplodia gonubiensis</i>	CMW 14077	AY639595	DQ103566	DQ458860	KU696359
<i>Lasiodiplodia gonubiensis</i>	CMW 14078	AY639594	DQ103567	EU673126	KU696358
<i>Lasiodiplodia gravistriata</i>	CMM 4564	KT250949	KT250950	N/A	N/A
<i>Lasiodiplodia gravistriata</i>	CMM 4565	KT250947	KT266812	N/A	N/A
<i>Lasiodiplodia hyalina</i>	CGMCC 3.17975	KX499879	KX499917	KX499992	KX499955
<i>Lasiodiplodia hormozganensis</i>	IRAN 1500C	GU945355	GU945343	KU887515	KU696361
<i>Lasiodiplodia hormozganensis</i>	IRAN 1498C	GU945356	GU945344	KU887514	KU696360
<i>Lasiodiplodia indica</i>	IBP 01	AP012032	N/A	N/A	N/A
<i>Lasiodiplodia iraniensis</i>	IRAN 1520C	GU945348	GU945336	KU887516	KU696363
<i>Lasiodiplodia iraniensis</i>	IRAN 1502C	GU945347	GU945335	KU887517	KU696362
<i>Lasiodiplodia jatrophicola</i>	CMM 3610	KF234544	KF226690	KF254927	N/A
<i>Lasiodiplodia jatrophicola</i>	CMW 36237	KU887121	KU886998	KU887499	KU696348
<i>Lasiodiplodia jatrophicola</i>	CMW 36239	KU887123	KU887000	KU887501	KU696349
<i>Lasiodiplodia laeliocattleyae</i>	CBS 130992	JN814397	JN814424	KU887508	KU696354

(Continued on next page)

^y Newly generated sequences are indicated in bold.

^z ITS, internal transcribed spacer; *RPB2*, RNA polymerase II; *tef1-α*, translation elongation factor-1α; *TUB*, β-tubulin, and N/A = not available.

Molecular characterization. DNA was extracted from mycelia grown on MEA plates using a CTAB plant genome DNA fast extraction kit (Aidlab Biotechnologies Co., Ltd). The internal transcribed spacer (ITS) ribosomal DNA region was amplified and sequenced with primers ITS-1 and ITS-4 (White et al. 1990), the RNA polymerase II gene (*RPB2*) with primers RPB2-LasF and RPB2-LasR (Cruywagen et al. 2017), the β -tubulin gene (*TUB*) with primers Bt2a and Bt2b (Glass and Donaldson 1995), and the translation elongation factor-1 α (*tef1*- α) with primers EF1-688F and EF1-986R (Alves et al. 2008; Carbone et al. 1999). PCR amplification and sequencing followed the protocol of Zhang et al. (2009).

DNA sequences of concatenated ITS, *RPB2*, *TUB*, and *tef1*- α loci were analyzed to investigate the phylogenetic relationships among *Lasiodiplodia* species using DNA sequences available from

GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) as well as the sequences generated herein (Table 1). Sequence alignment was performed with MEGA v. 6, and gaps were manually adjusted to optimize the alignment (Tamura et al. 2013). Analyses of maximum parsimony were performed with gaps being treated as missing data using PAUP v. 4.0b10 (Swofford 2002). Clade stability was evaluated in a bootstrap analysis with 1,000 replicates, random sequence additions with the maxtrees parameter set to 1,000, and other parameters left at default values as implemented in PAUP. Trees were viewed in TreeView v1.6.6 (Page 1996).

Pathogenicity testing. Mycelia and conidia of three isolates of *Lasiodiplodia vaccinii* (CGMCC3.19022, CGMCC3.19023, and CGMCC3.19024) and three of *B. dothidea* (CGMCC3.19259, CGMCC3.19258, and CGMCC3.19257) obtained in this study were

Table 1. (Continued from previous page)

Species	Isolate	GenBank accession number ^a			
		ITS	<i>tef1</i> - α	<i>TUB</i>	<i>RPB2</i>
<i>Lasiodiplodia lignicola</i>	CBS 134112	JX646797	KU887003	JX646845	KU696364
<i>Lasiodiplodia lignicola</i>	MFLUCC 11-0656	JX646798	N/A	JX646846	N/A
<i>Lasiodiplodia macrospora</i>	CMM 3833	KF234557	KF226718	KF254941	N/A
<i>Lasiodiplodia mahajangana</i>	CMW 27818	FJ900596	FJ900642	FJ900631	KU696366
<i>Lasiodiplodia mahajangana</i>	CMW 27801	FJ900595	FJ900641	FJ900630	KU696365
<i>Lasiodiplodia margaritacea</i>	CBS 122519	EU144050	EU144065	KU887520	KU696367
<i>Lasiodiplodia margaritacea</i>	CBS 122065	EU144051	EU144066	N/A	N/A
<i>Lasiodiplodia mediterranea</i>	CBS 137783	KJ638312	KJ638331	KU887521	KU696368
<i>Lasiodiplodia mediterranea</i>	CBS 137784	KJ638311	KJ638330	KU887522	KU696369
<i>Lasiodiplodia missouriana</i>	UCD 2193MO	HQ288225	HQ288267	HQ288304	KU696370
<i>Lasiodiplodia missouriana</i>	UCD 2199MO	HQ288226	HQ288268	HQ288305	KU696371
<i>Lasiodiplodia parva</i>	CBS 456.78	EF622083	EF622063	KU887523	KU696372
<i>Lasiodiplodia parva</i>	CBS 494.78	EF622084	EF622064	EU673114	KU696373
<i>Lasiodiplodia plurivora</i>	STE-U 5803	EF445362	EF445362	EF445362	EF445362
<i>Lasiodiplodia plurivora</i>	STE-U 4583	AY343482	EF445396	KU887525	KU696375
<i>Lasiodiplodia pontae</i>	CMM 1277	KT151794	KT151791	KT151797	N/A
<i>Lasiodiplodia pseudotheobromae</i>	CBS 116459	EF622077	EF622057	EU673111	KU696376
<i>Lasiodiplodia pseudotheobromae</i>	CGMCC 3.18047	KX499876	KX499914	KX499989	KX499952
<i>Lasiodiplodia pyriformis</i>	CBS 121771	EU101308	EU101353	KU887528	KU887528
<i>Lasiodiplodia pyriformis</i>	CBS 121770	EU101307	EU101352	KU887527	KU696378
<i>Lasiodiplodia rubropurpurea</i>	WAC 12535	DQ103553	DQ103571	EU673136	KU696380
<i>Lasiodiplodia rubropurpurea</i>	WAC 12536	DQ103554	DQ103572	KU887530	KU696381
<i>Lasiodiplodia sterculiae</i>	CBS 342.78	KX464140	KX464634	KX464908	KX463989
<i>Lasiodiplodia subglobosa</i>	CMM 4046	KF234560	KF254942	KF254944	N/A
<i>Lasiodiplodia subglobosa</i>	CMM 3872	KF234558	KF226721	KF254942	N/A
<i>Lasiodiplodia thailandica</i>	CPC 22795	KJ193637	KJ193681	N/A	N/A
<i>Lasiodiplodia thailandica</i>	CP 22755	KM006433	KM006464	N/A	N/A
<i>Lasiodiplodia theobromae</i>	CBS 111530	EF622074	EF622054	KU887531	KU696382
<i>Lasiodiplodia theobromae</i>	CBS 164.96	AY640255	AY640258	KU887532	KU696383
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19022	HM330318	HM330321	MH330324	HM330327
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19023	HM330319	HM330322	HM330326	HM330329
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19024	HM330320	HM330323	HM330325	HM330328
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19248	MK157131	MK157158	MK157149	MK157140
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19249	MK157132	MK157159	MK157150	MK157141
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19250	MK157133	MK157160	MK157151	MK157142
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19251	MK157134	MK157161	MK157152	MK157143
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19252	MK157135	MK157162	MK157153	MK157144
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19253	MK157136	MK157163	MK157154	MK157145
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19254	MK157137	MK157164	MK157155	MK157146
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19255	MK157138	MK157165	MK157156	MK157147
<i>Lasiodiplodia vaccinii</i>	CGMCC 3.19256	MK157139	MK157166	MK157157	MK157148
<i>Lasiodiplodia venezuelensis</i>	WAC 12539	DQ103547	DQ103568	KU887533	KU696384
<i>Lasiodiplodia venezuelensis</i>	WAC 12540	DQ103548	DQ103569	KU887534	KU887534
<i>Lasiodiplodia viticola</i>	UCD 2553AR	HQ288227	HQ288269	HQ288306	KU696385
<i>Lasiodiplodia viticola</i>	UCD 2604MO	HQ288228	HQ288270	HQ288307	KU696386
<i>Lasiodiplodia vitis</i>	CBS 124060	KX464148	KX464642	KX464917	KX463994

used to conduct pathogenicity testing. The pathogenicity test was performed on 4-year-old blueberry (cultivar O'Neal) plants obtained from seeds kept in a greenhouse at 28°C and 99% relative humidity. On healthy plants, 2-year-old twigs to be used for inoculation were surface disinfected with 75% ethanol for 1 min. The bark surface of each disinfected twig was stabbed 10 times with a sterilized inoculating needle within a region 5 mm in length to a depth of 2 mm (Lin et al. 2011). For mycelial inoculation, a 5-mm-diameter MEA plug with mycelia was taken from a 3-day-old colony and inoculated onto the wounded twigs. For conidial inoculation, 30 µl of suspension of 10^6 conidia ml⁻¹ obtained from pycnidia was crushed in sterile water and drop inoculated onto the wounded site (Espinoza et al. 2009). All of the inoculated twigs were covered with parafilm. Three replicates were conducted for each isolate. Noncolonized MEA plugs and sterile water were used as negative controls. Pathogenicity was determined by the length of the necrotic lesion caused by the tested isolates, which was measured 3 weeks after inoculation. Fungal isolates were reisolated from the infected tissue, and morphological characterization and DNA sequence comparisons were conducted to fulfill Koch's postulates. Mean comparisons were conducted using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$) in R (Version 3.2.2; R Inc.).

Temperature and pH tests. Because all of the 12 isolates of *L. vaccinii* showed similar growth characteristics, we used the type strain (CGMCC3.19022) to evaluate the effects of temperature and pH on colony growth on MEA plates. Temperatures tested ranged from 5 to 40°C at 5°C intervals (i.e., 5, 10, 15, 20, 25, 30, 35, and 40°C). To clarify the effect of pH on radial mycelial growth, MEA media were adjusted with 0.1 M NaOH and 0.1 M HCl to obtain

pH values from 2.0 to 12.0 at a pH 1.0 interval (i.e., 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0). A 5-mm-diameter mycelial plug was placed in the center of a 90-mm petri dish with MEA medium and incubated at 28°C in the dark, with three replicates for each treatment. The effects of pH and temperature on mycelial growth were determined by measuring the colony diameters after 24 and 48 h of incubation, and the data were converted to radial growth in millimeters (Xu et al. 2015). Data were analyzed in IBM SPSS Statistics (V22.0, IBM Inc.) to select the model that best fit the individual data points, and SPSS was used to confirm the selected model. The optimal temperature and pH value of the regression curves were calculated based on the regression equations generated by IBM SPSS Statistics.

Carbon and nitrogen source tests. To investigate the utilization of nitrogen and carbon sources, the representative strain of *L. vaccinii* (CGMCC3.19022) was incubated in the dark at 28°C on WA medium for 3 days. Czapek–Dox medium was used as the basic medium (sodium nitrate 3 g, dipotassium hydrogen phosphate 1 g, magnesium sulfate 0.5 g, potassium chloride 0.5 g, ferrous sulfate 0.01 g, sucrose 30 g, agar 20 g, and distilled water to complete 1,000 ml) (Czapek 1902, 1903; Dox 1910). The 30 g sucrose was replaced with 30 g fructose, galactose, glucose, maltose, sorbitol, sucrose, or xylose to test these compounds as carbon sources. The 3 g sodium nitrate was replaced with 3 g ammonium nitrate, ammonium sulfate, ammonium tartrate, glycine, tryptone, or yeast extract to determine growth on different nitrogen sources. A 5-mm-diameter WA plug of mycelia was transferred to the center of each sole carbon source medium and sole nitrogen source medium. Colony growth was determined by measuring the colony diameters after incubation for 24 and 48 h at 28°C in the dark, and the results were subsequently converted to

Fig. 1. *Lasiodiplodia vaccinii* (from ex type CGMCC3.19022). **A**, Colonies and reverse after 7 days on malt extract agar. **B**, Conidiomata formed on pine needles in culture after incubating for 3 weeks. **C**, Conidia developing on conidiogenous cells among paraphyses. **D and E**, Hyaline immature conidia. **F**, Germinating conidia and mature, one-septate conidia with longitudinal striations on the surface. Scale bars: **B** = 500 µm; **C to F** = 10 µm.

radial growth (Xu et al. 2015). Mean comparisons were conducted using Tukey's HSD ($\alpha = 0.05$) in R.

Results

Species characterization and identification. In total, 37 *Botryosphaeria* isolates, including 12 *Lasiodiplodia* isolates and 25 *Botryosphaeria* isolates, were obtained from 36 samples (of 100

samples) collected in this study. These 25 *Botryosphaeria* isolates had holoblastic, hyaline, subcylindrical conidiogenous cells that were narrowly fusiform or irregularly fusiform and one-celled conidia. The size of the conidia was 18 to 21×4 to $5 \mu\text{m}$, and it rarely formed a septum when senescent. All of these morphological characteristics agree with those of *B. dothidea* (Slippers et al. 2004). Furthermore, DNA sequence comparison of these 25 strains with the

Fig. 2. Maximum parsimony tree generated from sequence analysis of the concatenated internal transcribed spacer, RNA polymerase II gene, β -tubulin gene, and translation elongation factor-1 α dataset. The designated outgroup taxa are *Diplodia mutila* and *Diplodia seriata*. Bootstrap support values for maximum parsimony $\geq 60\%$ are shown above the nodes. The positions of the *Lasiodiplodia vaccinii* isolates are indicated in bold and red text. *Value $< 60\%$.

ex-type strain of *B. dothidea* (CBS 115476) indicated that the identities of both the ITS and the *tef1*- α region were 99.9%. The 25 *Botryosphaeria* isolates were thus identified as *B. dothidea*. Based on their morphology and the results of multigene phylogenetic analysis, the remaining 12 strains were identified as a new species of the genus *Lasiodiplodia*, which was named *L. vaccinii* (*L. vaccinii* Y Zhang et al. & L. Zhao sp. nov., Mycobank: MB 825125, etymology: from the genus *Vaccinium* in reference to the host genus).

Specimens examined were China, Beijing, Huiyuan Juice Blueberry Production Garden, blighted branches of *V. corymbosum*, 6 October 2017, Y. Zhang and L. Zhao (HMAS 255366, holotype; CGMCC3.19022, ex-type living culture).

Morphological characterization. Colonies of *L. vaccinii* growing on MEA were initially white with moderately dense, abundant, and straight aerial mycelia growing upward to the lid of the plate; they became pale olivaceous gray to olivaceous gray within 7 days, with the reverse side black-gray. Colonies (in 90-mm plates) reached a diameter of 31 mm after 24 h in the dark at 28°C and 84 mm after 48 h.

Sexual stage is unknown. Conidiomata were stromatic under greyish brown mycelia and formed on both pine needles and WA medium within 15 days; they were superficial or rarely semiimmersed, black, solitary, globose with a central ostiole with or without papilla, and 770 to 1,330 μ m in diameter. Paraphyses were filiform, hyaline, tip rounded, initially aseptate, becoming up to one to six septate when mature, rarely branched, extending above the developing conidia layer, ≤ 88 μ m in length, 2 to 5 μ m wide. Conidiophores were not observed. Conidiogenous cells were holoblastic, hyaline, discrete, smooth thin walled, (9 to) 11 to 17 (to 18) $\times$ 3 to 5 (to 6) μ m (average = 14.2×3.9 μ m, $n = 20$). Conidia were initially hyaline; wall was

1- μ m thick and ellipsoid to ovoid with rounded or slightly tapered apex, with a few turning brown with a median septum and longitudinal striations when aged: (18 to) 21 to 27 (to 31) $\times$ (11 to) 12 to 14 (to 16) μ m (average = 23.3×12.8 μ m, $n = 50$, L/W ratio = 1.8, range from 1.7 to 1.9); conidia usually germinated before septum formed (Fig. 1).

Molecular characterization. The alignment of the concatenated loci ITS, *RPB2*, *TUB*, and *tef1*- α from 90 strains (with *Diplodia mutila* and *Diplodia seriata* as the outgroup taxa) comprised 1,803 nucleotide sites, which included 1,406 constant sites, 303 parsimony-informative sites, and 94 parsimony-uninformative sites. Maximum parsimony analysis generated a total of 396 equally parsimonious trees. All trees were similar in topology and were not significantly different (not shown). One of the most parsimonious trees (tree length = 771, consistency index = 0.637, retention index = 0.875, rescaled consistency index = 0.557, homoplasy index = 0.363) is shown in Figure 2. In the phylogram, the 12 strains of *L. vaccinii* formed a terminal clade with 73% support (Fig. 2), which was sister to another terminal clade consisting of *Lasiodiplodia citricola* (Fig. 2). This result indicated that *L. vaccinii* is a new species, closely related to *L. citricola*.

Pathogenicity test. The three isolates of *L. vaccinii* (CGMCC3.19022, CGMCC3.19023, and CGMCC3.19024) tested in this study were pathogenic on the blueberry twigs when either mycelia or conidia were used as inocula. No symptoms were observed in the noninoculated control treatment (Table 2). Brown lesions appeared at the inoculated points 3 days after inoculation with mycelia and 5 days after inoculation with conidia. The diseased spots turned brown and lesion areas were ≤ 16 -cm long by 7 days after inoculation with both of the inocula. By 3 weeks after inoculation, the lengths of the brown necrotic lesions ranged from 8.5 to 26 cm for conidial inoculation and from 10 to 25 cm for mycelial inoculation. Over the same period, the infected xylem tissue turned light brown in both treatments. The lengths of lesions inoculated with mycelia or conidia showed no significant difference, although the average lesion length produced by a conidial suspension increased faster than that resulting from mycelial inoculation (Fig. 3). The necrotic discoloration length for *B. dothidea* was significantly shorter than that for *L. vaccinii* (Fig. 4 and Table 2).

Koch's postulates were performed by successful reisolation of fungal strains from all of the necrotic twigs inoculated with *B. dothidea* or *L. vaccinii*. The morphology and DNA sequences of the isolates retrieved from the inoculated twigs were consistent with those of the strains used for inoculations.

Effects of temperature and pH on mycelial growth. Mycelia of *L. vaccinii* grew on MEA in the temperature range from 15 to 40°C but did not grow at either 5 or 10°C after 48 h of incubation (Fig. 5). The fastest mycelial growth occurred at 30°C, reaching 38 mm after 24 h of incubation and 90 mm after 48 h of incubation, and the least amount of growth occurred at 15 and 40°C. No mycelial growth was observed at 15°C after 24 h of incubation, but the colony reached 10.8 mm after 48 h of incubation. The data conform to the regression equations $y = 33.21 - 7.41x + 0.486x^2 - 0.008x^3$ ($P < 0.05$, $R^2 = 0.899$) at 24 h of incubation and $y = 62.83 - 16.35x + 1.18x^2 - 0.02x^3$ ($P < 0.001$, $R^2 = 0.972$) at 48 h of incubation (x = temperature [degrees Celsius] and y = growth [colony diameter in millimeters]) (Fig. 5). Based on regression analysis, the optimum growth of *L. vaccinii* after both 24 and 48 h of incubation was estimated to occur at 30.3°C.

Mycelia of *L. vaccinii* grew on MEA in the pH range from 3.0 to 10.0 but did not grow at pH 2.0, 11.0, or 12.0 after 48 h of incubation. Mycelia grew most rapidly at pH 7.0 after 24 h of incubation, reaching 38.3 mm, followed by pH 6.0 and 5.0, which gave colony diameters of 35.8 and 34.9 mm, respectively. The mycelia fully covered the 90-mm dishes after 48 h of incubation at pH 5.0, 6.0, and 7.0, whereas they grew more slowly at pH values of 3.0, 4.0, 8.0, 9.0, and 10.0, attaining colony diameters of ≤ 80 mm after 48 h of incubation. The data fit the regression equations $y = -48.17 + 30.85x - 3.41x^2 + 0.10x^3$ ($P < 0.001$, $R^2 = 0.90$) after 24 h of incubation and $y = -124.83 + 79.33x - 8.53x^2 + 0.23x^3$ ($P < 0.001$, $R^2 = 0.93$) after 48 h of incubation (x = pH and y = growth [colony diameter in

Table 2. Pathogenicity of *Lasiodiplodia vaccinii* and *Botryosphaeria dothidea* on O'Neal blueberry plants using mycelia or conidia as inoculum 3 weeks after inoculation

Species	Isolate	Blueberry twigs inoculated with ^z	
		Mycelia (cm)	Conidia (cm)
<i>L. vaccinii</i>	CGMCC3.19022	15.33 $\pm$ 1.53 a	16.00 $\pm$ 7.94 a
<i>L. vaccinii</i>	CGMCC3.19023	17.33 $\pm$ 6.66 a	20.00 $\pm$ 5.10 a
<i>L. vaccinii</i>	CGMCC3.19024	15.67 $\pm$ 6.03 a	18.17 $\pm$ 8.89 a
<i>B. dothidea</i>	CGMCC3.19257	1.73 $\pm$ 0.46 b	2.93 $\pm$ 0.11 b
<i>B. dothidea</i>	CGMCC3.19258	1.80 $\pm$ 0.21 b	2.77 $\pm$ 0.25 b
<i>B. dothidea</i>	CGMCC3.19259	2.03 $\pm$ 0.31 b	3.10 $\pm$ 0.36 b
Noninoculated control	—	0.00 $\pm$ 0.00 c	0.00 $\pm$ 0.00 c

^z Data followed by different letters in each column are significantly different based on honest significant difference tests at the $P < 0.05$ level.

Fig. 3. Pathogenicity comparison for *Lasiodiplodia vaccinii* using mycelia or conidia as inocula (honest significant difference tests, $P < 0.05$). Values are means from three isolates (CGMCC 3.19022, CGMCC 3.19023, and CGMCC 3.19024). Vertical bars indicate standard errors.

millimeters)] (Fig. 6). Based on these regression equations, the optimal growth of *L. vaccinii* after 24 and 48 h of incubation was estimated to take place at pH 6.2.

Effects of carbon and nitrogen sources on mycelial growth. All of the seven carbon sources tested support *L. vaccinii* mycelial growth (Fig. 7). After 24 h of incubation, the utilization of sucrose was significantly greater than that for galactose and maltose, whereas there was no difference among fructose, glucose, and sorbitol, which were slightly less well utilized than the former three carbon sources (Fig. 7). The utilization of xylose was significantly lower than that of all other carbon sources tested (Fig. 7). After 48 h of incubation, however, sucrose utilization was significantly higher than that of galactose, maltose, and sorbitol, whereas there was no difference between the less well-utilized fructose and glucose (Fig. 7). Xylose had the lowest level of carbon utilization (Fig. 7). All of the nitrogen sources tested could support mycelial growth (Fig. 8). After 24 and 48 h of incubation, the utilization of yeast extract and tryptone was significantly higher than that of glycine, ammonium nitrate, and ammonium sulfate (Fig. 8). The utilization of ammonium tartrate was

significantly lower than that of all other nitrogen sources at both time points (Fig. 8).

Discussion

Phylogeny based on concatenated ITS, *RPB2*, *TUB*, and *tef1-α* DNA sequences showed that the 12 isolates of *L. vaccinii* formed a separate clade that was a sibling to other species of the genus *Lasiodiplodia* and closely related to *L. citricola* (Fig. 2). Morphologically, the paraphyses, conidiogenous cells, and conidia of *L. vaccinii* were all significantly (all $P < 0.001$, t test) narrower than those of *L. citricola* (Table 3). In particular, *L. vaccinii* could be distinguished from *L. citricola* by its short paraphyses (≤ 88 versus ≤ 120 μm). Furthermore, the conidia of *L. vaccinii* tended to remain hyaline, and only a small proportion (20% after 4 weeks) of the discharged conidia became pigmented with age, in contrast with those of *L. citricola* (80% after 4 weeks) (Abdollahzadeh et al. 2010; Dou et al. 2017a).

Pathogenicity tests were conducted on 4-year potted blueberry plants (with 2-year-old twigs) in a greenhouse with very similar conditions to those of the sampling sites. The results indicated that both

Fig. 4. Stem blight symptoms on blueberry caused by *Lasiodiplodia vaccinii* and *Botryosphaeria dothidea*. **A**, Death of the whole plant caused by *L. vaccinii* in the greenhouse. **B**, Stem blight caused by *L. vaccinii* in the greenhouse. **C**, Symptoms 3 weeks after inoculating with conidia of *B. dothidea*. Symptoms **D**, **2** and **E**, 3 weeks after inoculating with conidia of *L. vaccinii*. **F**, Symptoms on blueberry twig 3 weeks after inoculating with mycelium of *L. vaccinii*.

L. vaccinii and *B. dothidea* were pathogenic on blueberry twigs. *L. vaccinii* invaded the xylem and caused mortality of the whole branch within 3 weeks, showing the typical stem blight that occurred in the sampled greenhouse. By contrast, *B. dothidea*, however, turned the infected twig tissue dark brown, but the plants were still surviving 3 weeks after inoculation. Xu et al. (2015) also reported that *Lasiodiplodia* spp. were more virulent than *Botryosphaeria* spp. in blueberries.

A number of botryosphaeriaceous species have been reported to cause stem blight of blueberries, including *B. dothidea* and *L. theobromae* in southeastern United States (Creswell and Milholland

1988; Milholland 1972b; Witcher and Clayton 1962), *N. parvum* in California in the United States and in Mexico (Boyzo-Marín et al. 2016; Koike et al. 2014), *Neofusicoccum ribis* in Florida in the United States and in New Zealand (Tennakoon et al. 2018; Wright and Harmon 2010), and *Macrophomina phaseolina* in Serbia (Popović et al. 2018). In China, *B. dothidea*, *N. parvum*, *L. theobromae*, and *L. pseudotheobromae* have all been reported as causing blueberry stem blight (Wang et al. 2016; Xu 2016; Xu et al. 2015; Yu et al. 2012, 2013a, b). Among these species, *B. dothidea* was the most widely reported from temperate to subtropical areas of China, such as Liaoning, Shandong, Jiangsu, Zhejiang, Fujian, Yunnan, and Sichuan provinces (Xu 2016; Xu et al. 2015; Yu et al. 2012). *N. parvum* is mainly distributed in subtropical areas of China, such as Yunnan, Sichuan, Zhejiang, and Fujian (Xu 2016; Xu et al. 2015; Yu et al. 2013a, b). *L. theobromae* and *L. pseudotheobromae* have been mostly reported in tropical or subtropical areas of China, including Jiangsu and Zhejiang provinces, and only rarely in temperate areas of China, such as Shandong province (Wang et al. 2016; Xu 2016; Xu et al. 2015). The causal agents of stem blight caused by the Botryosphaeriaceae differ among locations worldwide, and this is probably because of the different environmental conditions, such as temperature and humidity, and the influence of management practices (Tennakoon et al. 2018).

The growth temperature for phytopathogenic fungi is generally from 10 to 35°C, with the optima from 20 to 30°C. For instance, the optimal growth temperature of *Penicillium cellarum* was reported as 22°C (Strausbaugh and Dugan 2017), that for *Diaporthe neotheicola* and *Diaporthe ambigua* was 25°C, the optimum for *Diaporthe* sp. was 22°C (Elfar et al. 2013), and that for *Phoma sorghina* was 20 to 25°C (Lin et al. 2015). The mycelium of *L. vaccinii*, however, grew from 15 to 40°C, with the optimal temperature being 30.3°C. Similar results have been reported for the type species of the genus *Lasiodiplodia*, *L. theobromae*, which showed growth at higher temperatures (15 to 40°C) and a higher optimal temperature (29 to 30°C) than most phytopathogens. Members of some other genera of the Botryosphaeriaceae, such as *N. ribis* and *B. dothidea*, have lower optimal (27 to 29°C) and growth (15 to 35°C) temperatures (Wright and Harmon 2010; Xu et al. 2015). The relatively high growth temperature ranges and optimal temperatures for *Lasiodiplodia* spp. may help to explain their dominant distribution in tropical or subtropical areas as well as the occurrence of *L. vaccinii* in the Beijing greenhouse, where the temperature and humidity are high.

Most phytopathogenic fungi grow optimally in a pH range between 5 and 6.5 (Cochrane 1958; Lin et al. 2015). The optimal pH value for *L. vaccinii* was from 5.0 to 7.0, although it could still grow slowly at pH values of 4.0 or 10.0. Similar results have been reported for *L. theobromae*, which could grow on media with a pH range from 4.0 to 10.0, with the optimal pH being in the range from 5.0 to 7.0 (Xu 2016). The optimal growth pH value of *B. dothidea*, however, has been found to range from 5.0 to 8.0, and it grew more slowly than *L. theobromae* or *L. vaccinii* (Xu 2016).

All of the seven carbon sources tested in this study could support the growth of *L. vaccinii*, although the utilization of xylose was less than that of all of the other six carbon sources. Xu et al. (2015), however, reported that *L. theobromae* has a high-xylose utilization rate (the diameter of the fungal colony reached 90 mm after 48 h of incubation). *L. vaccinii* could use all of the nitrogen sources tested in this study, which is similar to findings for *L. theobromae* (Xu 2016). Although *L. theobromae* can also use yeast extract, tryptone, ammonium sulfate, ammonium tartrate, ammonium nitrate, and glycine, its ability to utilize ammonium salts (ammonium sulfate, ammonium tartrate, and ammonium nitrate) was lower than that of *L. vaccinii* (Xu 2016).

L. vaccinii has been found only in greenhouse farms in the suburban Beijing area. Management practices, including better ventilation and lighting, might help to alleviate the damage resulting from stem blight caused by *L. vaccinii*. The distribution and host spectrum of *L. vaccinii* need additional study.

Fig. 5. Regression curves and equations for mycelial growth of *Lasiodiplodia vaccinii* after incubation for 24 and 48 h at 5, 10, 15, 20, 25, 30, 35, and 40°C on malt extract agar medium (x = temperature [degrees Celsius] and y = growth [colony diameter in millimeters]). Optimal mycelial growth temperature was estimated to be 30.3°C.

Fig. 6. Regression curves and equations for mycelial growth of *Lasiodiplodia vaccinii* after incubation for 24 and 48 h at pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0 on malt extract agar medium (x = pH and y = growth [colony diameter in millimeters]). Optimum mycelial growth pH was estimated to be 6.2.

Fig. 7. Effects of carbon source on growth of *Lasiodiplodia vaccinii* mycelium. Bars with different uppercase or lowercase letters represent significant differences after 24 and 48 h of incubation, respectively, according to honest significant difference tests at the $P < 0.05$ level.

Fig. 8. Effects of nitrogen source on growth of *Lasiodiplodia vaccinii* mycelium. Bars with different uppercase or lowercase letters represent significant differences after 24 and 48 h of incubation, respectively, according to honest significant difference tests at the $P < 0.05$ level.

Table 3. A morphological comparison of *Lasiodiplodia vaccinii* and *Lasiodiplodia citricola*

Species	Width of paraphyses (μm ; mean $\pm$ SD) ^z	Length of conidiogenous cells (μm ; mean $\pm$ SD)	Width of conidiogenous cells (μm ; mean $\pm$ SD)	Length of conidia (μm ; mean $\pm$ SD)	Width of conidia (μm ; mean $\pm$ SD)
<i>L. vaccinii</i>	2.98 $\pm$ 0.48 b	14.38 $\pm$ 2.10 a	3.89 $\pm$ 0.81 b	23.30 $\pm$ 2.56 a	12.80 $\pm$ 0.94 b
<i>L. citricola</i>	3.68 $\pm$ 0.62 a	14.18 $\pm$ 2.11 a	4.69 $\pm$ 0.74 a	23.18 $\pm$ 1.41 a	13.30 $\pm$ 0.67 a

^z SD, standard deviation.

Literature Cited

- Abdollahzadeh, J., Javadi, A., Goltapeh, E. M., Zare, R., and Phillips, A. J. 2010. Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. *Persoonia* 25:1-10.
- Alves, A., Crous, P. W., Correia, A., and Phillips, A. J. L. 2008. Morphological and molecular data reveal cryptic species in *Lasiodiplodia theobromae*. *Fungal Divers.* 28:1-13.
- Boyzo-Marin, J., Rebollar-Alviter, A., Silva-Rojas, H. V., and Ramirez-Maldonado, G. 2016. First report of *Neofusicoccum parvum* causing stem blight and dieback of blueberry in Mexico. *Plant Dis.* 100:2524.
- Carbone, I., Anderson, J. B., and Kohn, L. M. 1999. A method for designing primer sets for the speciation studies in filamentous ascomycetes. *Mycologia* 91:553-556.
- Chen, S. F., Li, G. Q., Liu, F. F., and Michailides, T. J. 2015. Novel species of Botryosphaeriaceae associated with shoot blight of pistachio. *Mycologia* 107: 780-792.
- Chen, S. F., Morgan, D. P., and Michailides, T. J. 2014. Botryosphaeriaceae and Diaporthaceae associated with panicle and shoot blight of pistachio in California, USA. *Fungal Divers.* 67:157-179.
- Cochrane, V. W. 1958. *Physiology of Fungi*. John Wiley & Sons, New York.
- Creswell, T. C., and Milholland, R. D. 1988. Spore release and infection periods of *Botryosphaeria dothidea* on blueberry in North Carolina. *Plant Dis.* 72: 342-346.
- Cruyagen, E. M., Slippers, B., Roux, J., and Wingfield, M. J. 2017. Phylogenetic species recognition and hybridisation in *Lasiodiplodia*: A case study on species from baobabs. *Fungal Biol.* 121:420-436.
- Czapek, F. 1902. Untersuchungen über die stickstoffgewinnung und eiweissbildung der pflanzen. *Beitr. Chem. Physiol. Pathol.* 1:538-560.
- Czapek, F. 1903. Untersuchungen über die stickstoffgewinnung und eiweissbildung der schimmelpilze. *Beitr. Chem. Physiol. Pathol.* 3:47-66.
- Dou, Z. P., He, W., and Zhang, Y. 2017a. Does morphology matter in taxonomy of *Lasiodiplodia*? An answer from *Lasiodiplodia hyalina* sp. nov. *Mycosphere* 8: 1014-1027.
- Dou, Z. P., He, W., and Zhang, Y. 2017b. *Lasiodiplodia chinensis*, a new holomorphic species from China. *Mycosphere* 8:521-532.
- Dox, A. W. 1910. The Intracellular Enzymes of *Penicillium* and *Aspergillus*: With Special Reference to Those of *Penicillium camemberti*. USDA Bureau of Animal Industry, Washington, DC.
- Elfarr, K., Torres, R., Díaz, G. A., and Latorre, B. A. 2013. Characterization of *Diaporthe australafricana* and *Diaporthe* spp. associated with stem canker of blueberry in Chile. *Plant Dis.* 97:1042-1050.
- Espinoza, J. G., Briceño, E. X., Chávez, E. R., Úrbez-Torres, J. R., and Latorre, B. A. 2009. *Neofusicoccum* spp. associated with stem canker and dieback of blueberry in Chile. *Plant Dis.* 93:1187-1194.
- Evans, E. A., and Ballen, F. H. 2014. An overview of US blueberry production, trade, and consumption, with special reference to Florida. University of Florida, IFAS, Extension, FE952.
- 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.
- Koike, S. T., Rooneylatham, S., and Wright, A. F. 2014. First report of stem blight of blueberry in California caused by *Neofusicoccum parvum*. *Plant Dis.* 98:1280.
- Kong, C. S., Qiu, X. L., Yi, K. S., Yu, X. F., and Yu, L. 2010. First report of *Neofusicoccum vitifusiforme* causing blueberry blight of blueberry in China. *Plant Dis.* 94:1373.
- Li, Y. D., Sun, H. Y., and Chen, L. 2016. China blueberry industry development report. China Fruits. 5:1-10.
- Lin, Y. L., Huang, L. L., Suolang, L. M., Gao, X. N., Chen, Y. C., and Kang, Z. S. 2011. A rapid laboratory evaluation system for apple ring rot. *Acta Phytopathol. Sin.* 38:37-41.
- Lin, Z. Y., Wei, J. J., Zhang, M. Q., Xu, S. Q., Guo, Q., Wang, X., Wang, J. H., Chen, B. S., Que, Y. X., Deng, Z. H., Chen, R. K., and Powell, C. A. 2015. Identification and characterization of a new fungal pathogen causing twisted leaf disease of sugarcane in China. *Plant Dis.* 99:325-332.
- Michailides, T. J., and Morgan, D. P. 1992. Effects of temperature and wetness duration on infection of pistachio by *Botryosphaeria dothidea* and management of disease by reducing duration of irrigation. *Phytopathology* 82:1399-1406.
- Michailides, T. J., and Morgan, D. P. 2004. Panicle and shoot blight of pistachio: A major threat to the California pistachio industry. *APSnet Feature*. doi:10.1094/APSnetFeature-2004-0104
- Milholland, R. D. 1972a. Factors affecting sporulation and infection by the blueberry stem canker fungus, *Botryosphaeria corticis*. *Phytopathology* 62: 137-139.
- Milholland, R. D. 1972b. Histopathology and pathogenicity of *Botryosphaeria dothidea* on blueberry stems. *Phytopathology* 62:654-660.
- Page, R. D. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* 12:357-358.
- 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.
- 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.
- Phillips, A. J. L., Oudemans, P. V., Correia, A., and Alves, A. 2006. Characterisation and epitypification of *Botryosphaeria corticis*, the cause of blueberry cane canker. *Fungal Divers.* 21:141-155.
- Popović, T., Blagojević, J., Aleksić, G., Jelušić, A., Krnjajić, S., and Milovanović, P. 2018. A blight disease on highbush blueberry associated with *Macrophomina phaseolina* in Serbia. *Can. J. Plant Pathol.* 40:121-127.
- Shi, H. Z., and Liu, H. M. 2009. The current research situation of blueberry. *J. Jiangsu For. Sci. Technol.* 36:48-51.
- Slippers, B., Crous, P. W., Denman, S., Coutinho, T. A., Wingfield, B. D., and Wingfield, M. J. 2004. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* 96:83-101.
- 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, B. J. 2004. Susceptibility of southern highbush blueberry cultivars to *Botryosphaeria* stem blight. *Small Fruits Rev.* 3:193-201.
- Strausbaugh, C. A., and Dugan, F. 2017. A novel *Penicillium* sp. causes rot in stored sugar beet roots in Idaho. *Plant Dis.* 101:1781-1787.
- Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4.0b10. Sinauer Associates, Sunderland, MA.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30: 2725-2729.
- Tang, W., Ding, Z., Zhou, Z. Q., Wang, Y. Z., and Guo, L. Y. 2012. Phylogenetic and pathogenic analyses show that the causal agent of apple ring rot in China is *Botryosphaeria dothidea*. *Plant Dis.* 96:486-496.
- Tennakoon, K. M. S., Ridgway, H. J., Jaspers, M. V., and Jones, E. E. 2018. Factors affecting *Neofusicoccum ribis*, infection and disease progression in blueberry. *Eur. J. Plant Pathol.* 151:87-99.
- van Niekerk, J. M., Crous, P. W., (Ewald) Groenewald, J. Z., Fourie, P. H., and Halleen, F. 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia* 96:781-798.
- Wang, J. N., Zhao, H. H., Ying-Ying, Y. U., Xiao-Dong, L. I., Liang, C., and Bao-Du, L. I. 2016. The pathogen causing *Lasiodiplodia* twig blight of blueberry. *Mycosystema* 35:657-665.
- 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. Smitsky, and T. J. White, eds. Academic Press, New York, NY.
- Witcher, W., and Clayton, C. N. 1962. Blueberry stem blight caused by *Botryosphaeria dothidea*. *Phytopathology* 53:705-712.
- Wright, A. F., and Harmon, P. F. 2009. First report of *Lasiodiplodia theobromae* associated with stem blight of southern highbush blueberries in Florida. *Plant Dis.* 93:962.
- Wright, A. F., and Harmon, P. F. 2010. Identification of species in the Botryosphaeriaceae family causing stem blight on southern highbush blueberry in Florida. *Plant Dis.* 94:966-971.
- Xu, C. N. 2016. Etiology, Molecular Detection and Control of Blueberry Stem Blight in China. Hebei Agricultural University, Baoding, China.
- Xu, C. N., Zhang, H. J., Zhou, Z. S., Hu, T., Wang, S. T., Wang, Y. N., and Cao, K. Q. 2015. Identification and distribution of Botryosphaeriaceae species associated with blueberry stem blight in China. *Eur. J. Plant Pathol.* 143: 737-752.
- Yu, L., Impaprasert, R., Zhao, J. R., Xu, S. G., and Wu, X. 2013a. Stem dieback of highbush blueberries caused by *Neofusicoccum parvum* in China. *New Dis. Rep.* 27:3.
- Yu, L., Rasisara, I., Xu, S. G., Wu, X., and Zhao, J. R. 2012. First report of stem blight of blueberry caused by *Botryosphaeria dothidea* in China. *Plant Dis.* 96: 1697.
- Yu, L., Zhao, J. R., Impaprasert, R., Xu, S. G., and Wu, X. 2013b. Identification of the pathogen causing twigs and stem dieback in blueberry. *Acta Phytopathol. Sin.* 43:421-425.
- Zhang, Y., Wang, H. K., Fournier, J., Crous, P. W., Jeewon, R., Pointing, S. B., and Hyde, K. D. 2009. Towards a phylogenetic clarification of *Lophiostoma Massarina* and morphologically similar genera in the Pleosporales. *Fungal Divers.* 38:225-251.