Identification and Characterization of *Botryosphaeria* spp. Causing Gummosis of Peach Trees in Hubei Province, Central China

Fan Wang[1]

Lina Zhao[1]

Guohuai Li[1]

Junbin Huang[2]

Tom Hsiang[3]

[1]Key Laboratory of Horticultural Plant Biology (Huazhong Agricultural University, Wuhan 430070, Hubei, China), Ministry of Education, China

Corresponding author: Guohuai Li

E-mail: liguohuai@mail.hzau.edu.cn

[2]The Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan 430070, Hubei, China

[3]School of Environmental Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada

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ABSTRACT

Peach (*Prunus persica*) is one of the most important and widely grown fruit trees in China; however, perennial gummosis on trunks and branches is a major problem in peach orchards of Hubei Province, one of the most important peach production areas of China. In order to identify the gummosis-causing agents, diseased trunks and branches were collected from 11 peach orchards in Hubei Province. Fungal isolates were obtained from these samples, yielding three species: *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*), *B. rhodina* (anamorph *Lasiodiplodia theobromae*), and *B. obtusa*

(anamorph *Diplodia seriata*). They were identified based on conidial morphology and cultural characteristics, as well as analyses of nucleotide sequences of three genomic regions: the internal transcribed spacer region (ITS), a partial sequence of the β-tubulin gene and the translation elongation factor 1-α gene (EF1-α). *Fusicoccum aesculi* was found in all 11 orchards, but *L. theobromae* was found only in Shayang county in the Jingmen region and *D. seriata* only in Gong'an county in the Jingzhou region. Via artificial inoculation using mycelia on wounded twigs or branches, these three species were all found to be pathogenic, causing dark lesions on the twigs and branches, and sometimes gum exudation from diseased parts. Isolates of *L. theobromae* were the most virulent, and caused the largest lesions and most copious gummosis, and *D. seriata* had less gum than the other two species. This report represents the first description of *L. theobromae* and *D. seriata* as causal agents of gummosis on peach in China.

Additional keywords: *Prunus persica*, gummosis, *Botryosphaeria*, morphology

The peach (*Prunus persica* L.) industry in Hubei Province presently comprises over 46,000 ha and is an important agricultural commodity in the province, producing an annual crop valued at over 134 million U.S. dollars. Fungal gummosis on trunks and branches of peach trees has become a growing threat to the peach industry in this province and throughout southern China, but is much less common in northern China. Disease symptoms are associated with lenticels, and include necrotic lesions on bark, and gum formation on trunks, scaffold limbs and branches (46,47). Gummosis also frequently occurs on other stone fruits such as apricot, almond, plum and cherry, and reduces tree growth and fruit yield, especially in susceptible cultivars (6).

Botryosphaeria spp. are found on a great number of hosts and have a wide geographical distribution (5,20,32,46). Since the genus was established in 1863 (Cesati & De Notaris) (15), based

on the type species *B. dothidea* (Moug. ex Fr.) Ces. & De Not. (4), different species of *Botryosphaeria* have been well characterized as canker-causing agents in numerous woody and non-woody plants (6,21,32,34,45). The genus is undergoing taxonomic revision, and a recent study redefined the genus as being restricted to *B. dothidea* and *B.corticis* (19).

Although the teleomorphic names are preferably used, the sexual structures are usually rare in the field, and hard to produce in culture. The anamorphic spore-producing structures of these fungi are much more frequently encountered and play an important role in their identification. Numerous anamorphs have been assigned to *Botryosphaeria* spp., and some studies based on DNA sequence analysis have demonstrated a clear phylogenetic boundary between species in *Fusioccum*-like and *Diplodia*-like anamorphs (20). *Fusicoccum* anamorphs have been associated with hyaline conidia, and *Diplodia* anamorphs with pigmented conidia (20). Phillips *et al*. determined that the anamorph of *B. dothidea* is *Fusicoccum aesculi* Corda (31), which has hyaline and fusiform conidia. Among the species with pigmented conidia, *B. rhodina* (Cooke) Arx always groups separately from other the species (20,27,29,53). *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl., the anamorph of *B. rhodina*, has much larger conidia than the other *Diplodia* species (32). The anamorphic state of *B. obtusa* (Schwein.) Shoemaker was found to be *Diplodia seriata* De Not. (30). To date, three *Botryosphaeria* spp. have been reported to cause gummosis symptoms in peach, including *B. dothidea* (anamoph *Fusicoccum aesculi*), *B. rhodina* (anamorph *Lasiodiplodia theobromae*), and *B. obtusa* (anamorph *Diplodia seriata*) (8,9,18,47).

Among the *Botryosphaeriaceae*, *F. aesculi* is one of the most common species and widespread across the world. Numerous economically important plants were found to be infected by the pathogen, such as olive, grapevine, apple, *Pinus* spp., kiwifruit, pistachio and sweet osmanthus (28,31,51). *Lasiodiplodia theobromae* is a common endophyte and opportunistic pathogen on more than 500 tree

species in the tropics and subtropics (12). It is the causal agent of cankers, dieback of ornamental plants, vegetable crops, and perennial fruit and nut trees (33). *Diplodia seriata* has been reported in many countries and recognized as an important pathogen of stone and pome fruit trees, causing cankers, leaf spots and fruit black rot (9,10,11,39), and in grapevine, causing cankers and brown streaking in wood (14,24,38,43,44).

The aim of the present study was to identify and characterize *Botryosphaeria* spp. associated with gummosis symptoms of peach trees in central China based on morphological, molecular and pathological characteristics.

MATERIALS AND METHODS

Field observations, disease symptoms and fungal isolation. Field surveys were carried out throughout 2009 in 11 peach orchards of Hubei Province, China. The appearance of gummosis symptoms in peach trees was characterized and data on the location assembled. In all, 136 gummosis samples were collected from different peach cultivars and brought back to the laboratory.

After surface disinfestation of lesions on woody tissue with 0.1% mercuric chloride for 40 s or 5% sodium hypochlorite for 1 min, followed by autoclaved water wash three times, small blocks (9 mm^2) of diseased bark were aseptically transferred to 2% potato dextrose agar (PDA) plates. Cultures were incubated at 25°C until fungal growth was observed. *Botryosphaeria* isolates were purified by single spore culturing (17) prior to use in experiments.

Morphological characterization. *Botryosphaeria* spp. isolated from gummosis samples were identified tentatively based on colony morphology and conidial characteristics reported in previous studies (25,26,30,31). *Botryosphaeria* isolates first were divided by colony morphology (color and

aerial hyphal growth), and 10 isolates were examined for conidial morphology. Selected cultures were transferred onto PDA or oatmeal agar (3) at 25°C. Then conidial morphology (length, width, color, and presence or absence of septa) from pycnidia of each isolate was determined for each isolate using a compound microscope camera (Nikon Digital Sight DS-U1) (Table 1). Mean, standard deviation and 95% confidence intervals were calculated from measurements of 50 conidia for each isolate. Conidial color, shape, and presence or absence of septa were also recorded.

Botryosphaeria isolates selected for conidial measurements were also used to determine the colony growth characteristics of different species. A 6-mm-diameter plug from the growing margin of a 3-day-old colony was placed in the center of a 90-cm-diameter PDA petri dish, and five replicates of each isolate were incubated at 25°C under light. Colony diameter was measured after 2, 3 and 4 days of incubation, and data were converted to radial growth, which was statistically compared among isolates.

DNA extraction, amplification and multigene phylogenetic analysis. Genomic DNA from 13 *Botryosphaeria* isolates (Table 1) was extracted from fungal mycelia following Alves et al. (1). The ITS region was amplified using the primers ITS1 and ITS4 (49). The primers Bt2a and Bt2b (22) of partial sequence of the β-tubulin (Bt) gene were from Sakalidis (37), and the EF1-α primers EF1-728F and EF1-986R (13) were from Phillips et al. (29). All primers were synthesized by BGI (Beijing, China). Each polymerase chain reaction (PCR) was carried out with 5 U/μL rTaq DNA polymerase, 2.5 mM nucleotide mix, 2.5 mM $MgCl_2$ and 10× buffer supplied by Takara company (Dalian, China). Temperature profiles for PCR with ITS and β-tubulin primers were as follows: an initial denaturation for 2 min at 94°C, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 90 s. Cycling conditions for EF1-α primers were as follows: initial

preheating at 95°C for 3 min, and then 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 5 min. The PCR amplification products were visualized on agarose gels, and some samples purified and selected for sequencing by BGI using forward and reverse primers.

Sequences were edited and assembled using DNAMAN (version 5.2.2; Lynnon Biosoft). The consensus sequences were compared by Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) NR database, and top matches were downloaded. As well, sequences annotated as *Botryosphaeria* spp. in GenBank were included in our phylogenetic analyses (Table 2). Sequences were aligned using CLUSTALX version 1.83 (42) with the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25%) following Alves et al (2).

Alignments were checked and manual adjustments were made where necessary. Alignment gaps were treated as missing data (43), and CLUSTALX was used to generate preliminary neighbor-joining trees which were viewed using Treeview (version 1.6.1; University of Glasgow, Glasgow, UK). Separate analyses were run for the ITS dataset alone, β-tubulin dataset alone, and EF1-α dataset alone, and a combination of the three gene datasets using PAUP* (version 4.0b10; Sinauer Associates. Inc., Sunderland, MA; 38). *Guignarda philoprina* and *Mycosphaerella pini* were used as the outgroups for all analyses (43). All characters were considered unordered and of equal weight. Maximum parsimony for all analyses was performed using the heuristic search option (branch swapping NNI), and 1,000 random addition sequences replicates. Bootstrap values were evaluated using 1,000 replicates to test branch strength. Tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) also were recorded for all

analyses.

Pathogenicity tests. Pathogenicity of selected isolates (Table 1) was tested on healthy twigs and branches of peach trees. The isolates were grown on PDA at 25°C for 5 days prior to inoculation. An initial pathogenicity test was conducted on 1-year-old twigs of peach cv. Shuguang. Twigs were wounded by a sterilized needle, and a 6-mm-diameter mycelium agar plug was placed on the wound. All inoculated twigs were placed in a plastic container, and the container was covered with plastic film, with five twigs per fungal isolate, and three inoculation sites for each twig. Five twigs were inoculated with 6-mm-diameter fresh PDA agar plugs as control. Disease symptoms were checked daily for a week following inoculation. Furthermore, vascular discoloration induced by *F. aesculi*, which was the most common *Botryosphaeria* species from peach in previous studies, was assessed at the same time.

A second pathogenicity test, using 10 *Botryosphaeria* isolates including eight isolates of *F. aesculi*, one *L. theobromae* and one *D. seriata*, was conducted on 7-year-old cv. Shuguang trees in the field. In all, 90 green twigs of approximately 30 cm in length and 90 3-year-old branches in peach orchards of Huazhong Agricultural University were used. Three wounds were made for each twig or branch with a sterilized needle. After inoculating with 6-mm-diameter mycelial plugs on the wounds, the inoculated area was wrapped immediately with six layers of cheesecloth saturated with sterile water and covered with polyethylene film to maintain high humidity (48). Controls were inoculated with pieces of uncolonized PDA. Two weeks after inoculation, visual assessment was made for each inoculation point of visible discoloration in the surrounding tissue.

Analyses of variance (ANOVA) in SAS (version 8.1; SAS Institute, Cary, NC) was used to assess differences in the rate of expansion of vascular discoloration in the first detached twig

pathogenicity tests. Disease incidence of 10 isolates in the second pathogenicity test was also analyzed by ANOVA and a significant treatment effect was found, these means were compared with the test of least significant difference ($P \leq 0.05$).

RESULTS

Field observation, fungal isolate, and data analysis. A total of 881 trees from 11 peach orchards were surveyed in Hubei Province, China, covering an area 300 km by 260 km (Fig. 1). Gummosis was observed in 818 of 881 trees examined. A total of 68 isolates from 136 samples with colony and conidial morphology resembling species of *Botryosphaeria* were obtained. Other species isolated such as *Alternaria* spp., *Fusarium* spp., and *Pestalotia* spp. were not considered further in this study.

Morphological characterization. All selected isolates developed white colonies which turned to gray or greenish to black after 14 days on PDA. Out of 10 isolates, two developed abundant aerial hyphae and two had scarce aerial growth, and the remainder were in between. Based on colony and conidial morphology, three different fungal groups were differentiated (Table 3; Fig. 2). The first fungal group formed white to olive-green colonies that developed moderate aerial growth, and conidia were fusiform and hyaline. The second fungal group was characterized by abundant aerial and fast-growing mycelium, with conidia that were subovoid, thick-walled, initially hyaline and aseptate, remaining hyaline for a long time, and finally becoming dark brown and one-septate. The third fungal group also produced dark-green colonies, but sparse mycelia. For this last group, single, small, dark-brown and black pycnidia were observed in 7-day-old cultures, and conidia were initially hyaline and aseptate, becoming dark-brown with age. Based on previous reports (31,32, 39,41,43,44), isolates from Hubei Province were identified as *Fusicoccum aesculi* (Figs. 2a, b and c), *Lasiodiplodia*

theobromae (Figs. 2d, e and f) or *Diplodia seriata* (Figs. 2g, h and i), corresponding to the groups above. On PDA at 25 °C, the radial growth rate of isolates of *L. theobromae* (6.5 ± 0.3 cm/day) was significantly higher than *F. aesculi* (4.0 ± 0.3 cm/day) or *D. seriata* (4.0 ± 0.2 cm/day).

Phylogenetic analysis. ITS, β-tubulin, and EF1-α sequences of *F. aesculi*, *L. theobromae* and *D. seriata* from peach trees in Hubei Province (Table 1) were aligned with GenBank sequences of *Botryosphaeria* spp. from different hosts and other countries (Table 2). After alignment, a partition homogeneity test showed a value of P = 0.06, indicating that the ITS, β-tubulin, and EF1-α datasets were congruent (P > 0.05) and could be combined in a single phylogenetic analysis. The combined dataset consisted of 1,182 characters, of which 638 were constant, 302 were parsimony uninformative, and 242 were parsimony informative. The maximum parsimony analyses yielded one most parsimonious tree (length = 774, CI = 0.870, RI = 0.932, RC =0.810, and HI =0.130; Fig. 3). The combined data set phylogenetic tree included two well-separated clades, one of which had two subclades. *Botryosphaeria* spp. with *Fusicoccum*-type conidia (hyaline and thin walled) composed a highly supported clade (100% bootstrap support) with *F. aesculi*. *Botryosphaeria* spp. with *Diplodia*-type conidia (pigmented and thick walled) formed a well-separated clade (100% bootstrap support). Within this clade, *L. theobromae* and *D. seriata* isolates formed two strongly supported subclades, both with with 100% bootstrap values (Fig. 3). Analyses of the ITS, β-tubulin, and EF1-α datasets separately yielded similar tree topologies (trees not shown) as the combined dataset, and the only differences between trees generated from the different datasets were changes in the positions of some isolates within one of the clades. Results from both the combined and single datasets of the ITS, β-tubulin, and EF1-α DNA sequences verified the morphological identification of *F. aesculi*, *L. theobromae* and *D. seriata* from peach trees in Hubei Province, central China.

Pathogenicity tests. All 17 *Botryosphaeria* isolates tested caused brown discoloration in 1-year-old detached twigs of peach cv. Shuguang. The pathogens were reisolated from inoculated twigs, while the peach twigs in the control set remained non-symptomatic and did not yield the pathogens. All 15 isolates identified as *F. aesculi* obtained from peach trees were pathogenic on peach, with development of lesions and necrotic tissue, and sometimes gum exudation from infected tissues wound within a week of inoculation. The rate of vascular discoloration rate in detached green twigs differed significantly (P = 0.05) between the strains with the fastest rate at 13.8 mm/day compared to the slowest at 3.8 mm/day (Table 4).

In a second pathogenicity test on 1-year-old intact twigs, 10 isolates including eight isolates of *F. aesculi*, one *L. theobromae* and one *D. seriata* were found to be pathogenic on peach cv. Shuguang trees in inoculated wounded tissue trials. Brown lesions with incidence of 70 to 90% were observed for the different *F. aesculi* isolates. Reddish brown canker lesions caused by *F. aesculi*, *L. theobromae* and *D. seriata* in living twigs were, respectively, 1.7 ± 1.7, 5.8 ± 2.0 and 0.3 ± 0.4 mm in length. On wound-inoculated 3-year-old branches, *L. theobromae* was the most virulent, with cankers 10.3 ± 4.4 mm in diameter and caused the largest lesions (up to 15 mm) and the most copious gum production, while *D. seriata* caused much less gum production and the smallest lesions compared to the other species.

DISCUSSION

This study represents the first attempt to characterize the species of *Botryosphaeria* associated with peach tree gummosis in Hubei Province, China, with integration of morphological, pathological and molecular data. Preliminary identifications were based on cultural and conidial morphology. And

then ITS, β-tubulin and EF1-α sequence data were used to confirm *Botryosphaeria* species identification. The present study has shown that three *Botryosphaeria* anamorphs, *Fusicoccum aesculi*, *Lasiodiplodia theobromae* and *Diplodia seriata*, are associated with peach tree gummosis in Hubei Province, central China.

The three species of *Botryosphaeria* found in Hubei Province have been reported in other peach growing areas worldwide. Peach tree gummosis was first noticed in the 1970s in Fort Valley, Georgia, USA, and the casual agent was first identified as *B. dothidea* (47). A subsequent report found *B. dothidea*, *B. rhodina* and *B. obtusa* causing peach tree gummosis in Georgia (8). In previous studies, Chen (16) first reported the occurrence of *B. dothidea* causing gummosis of peach trees in Jiangsu Province, China, and Wu et al (50) found *Physalospora persicae* caused peach blister canker, which occurred in 1- or 2-year-old branches in Shanghai Municipality. This is the first report of *L. theobromae* or *D. seriata* causing peach tree gummosis in China.

Pathogenicity tests conducted on 1-year-old twigs and 3-year-old branches of cv. Shuguang confirmed that *F. aesculi*, *L. theobromae* and *D. seriata* isolates from Hubei Province were pathogenic. In these tests, *L. theobromae* isolates produced much larger lesions and much more gummosis than the other two species. These results are consistent with a previous study conducted in Georgia, USA in which *L. theobromae* was found to be the most virulent species on peach trees (10). However, in the current study, this species was only found in Shayang county of Jingmen region, Hubei Province (Fig. 1). *Lasiodiplodia theobromae* is common in the tropics, and is prevalent in areas with high temperatures (32). Hubei Province is in central reaches of the Yangtze River, and has a warm, humid subtropical climate, but with cool winter conditions down to freezing temperatures (52), which might be less favorable for this species.

In this study, *F. aesculi* was the dominant species associated with peach tree gummosis in central

China, which differed from the report by Britton and Hendrix (8) showing that *D. seriata* was the most common species from natural cankers. Pusey (35,36) evaluated fungal colonization of outer bark, inner bark, and xylem of 1-year-old peach trees after inoculation without wounding, and found that *F. aesculi* was consistently dominant in diseased inner bark associated with lenticels, which is consistent with Weaver's results (47,48) that only *F. aesculi* has been shown to cause the lenticel-associated symptoms of peach gummosis in non-wounding inoculation studies; and that *L. theobromae* and *D. seriata* inhabited only dead outer bark as saprophytes, based on high recovery rates from outer bark and rare isolation from inner bark (35,36). The present study did not separate the outer bark from the inner bark for isolation, but the results revealed the dominance of *F. aesculi*, which is consistent with the previous work (35,36). In our pathogenicity tests, wounded twigs and branches were inoculated with *Botryosphaeria* spp., and the three species were pathogenic, similar to Britton and Hendrix's findings (8,9), even though *B. dothidea*, possibly even host-specialized strains (36), has been the only pathogen so far shown to cause the lenticel-associated symptoms. Pusey maintained the cankers induced by *L. theobromae* and *D. seriata* as being associated with wounds, but not natural openings such as lenticels (35). However, the two species may play roles as secondary organisms to invade older infections, or become opportunistic by infecting through wounds, so the risk by these fungi is present with abiotic stresses, and this may occur in summers with very high temperatures and moisture in central and southern China.

The shape, size, presence of septum, and pigment of conidia in combination with molecular data can be useful for identifying fungi, especially where teleomorphic connections have not been found or are rare (22). However, characteristics of cultures and conidia can vary. For instance, the isolates of *D. seriata* produced moderate aerial mycelium growth in other study (44), whereas *D. seriata* in this study formed colonies with sparse mycelia. Therefore, DNA sequence comparisons may be useful to

confirm the identification of anamorphs of *Botryosphaeria*.

Previous work has demonstrated the difficulty of controlling peach tree fungal gummosis caused by species of *Botryosphaeria*. Beckman and Reilly tested the susceptibility of 25 peach cultivars to *B. dothidea*, and showed that no cultivar was immune, and that 'Summergold' was the most susceptible and 'Redskin' the least. They believed drought conditions and isolated location resulted in the low disease incidence (7). In the current study, *Prunus persica* cv. Shuguang was found to be susceptible to infection via wounding and artificial inoculation by all three *Botryosphaeria* species. Infection studies with more diverse cultivars are needed to better characterize possibly resistant lineages. Studies designed to rapidly detect peach tree gummosis caused by *Botryosphaeria* spp. and to screen for peach germplasm resistance in China are currently underway.

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Table 1. *Botryosphaeria* spp. isolates from *Prunus persica* from Hubei Province, central China used in the phylogenetic study

Isolate	Identity[b]	Host cultivar[c]	Origin	GenBank accession number[a]		
				ITS	β-tubulin	EF1-α
GA-221	*Fusicoccum aesculi*	n/a	Gong'an	HQ660452	HQ660476	HQ660478
GA-722	*F. aesculi*	n/a	Gong'an	HQ660453	HQ660475	HQ660479
LHKA-221	*F. aesculi*	Zhongyou No. 5	Laohekou	HQ660454	HQ660473	HQ660480
SZ-422	*F. aesculi*	Shuguang	Suizhou	HQ660455	HQ660472	HQ660481
SZ-821	*F. aesculi*	Shuguang	Suizhou	HQ660456	HQ660471	HQ660482
WH-51	*F. aesculi*	Zhongyou No. 5	Wuhan	HQ660457	HQ660470	HQ660483
XG-221	*F. aesculi*	Daguan No. 3	Xiaogan	HQ660458	HQ660469	HQ660484
XG-52	*F. aesculi*	Daguan No. 3	Xiaogan	HQ660459	HQ660468	HQ660485
XNHG-12WR	*F. aesculi*	Jingchun	Xianning	HQ660460	HQ660467	HQ660486
XNHG-91WR	*F. aesculi*	Jingchun	Xianning	HQ660461	HQ660466	HQ660487
ZY-713	*F. aescuil*	Zaofengwang	Zaoyang	HQ660462	HQ660465	HQ660488
GA-422	*Diplodia seriata*	n/a	Gong'an	HQ660463	HQ660477	HQ660489
JMB-122	*Lasiodiplodia theobromae*	n/a	Jingmen	HQ660464	HQ660474	HQ660490

[a] ITS = internal transcribed spacer and EF1-α = elongation factor.

[b] *Botryosphaeria* spp. from peach from Hubei Province were determined based on morphology and phylogenetic analyses.

[c] n/a = Not available.

Table 2. Sequences of *Botryosphaeria* used in the phylogenetic study[a]

Isolate[b]	Species	Host	Location	GenBank No.			Reference
				ITS	β-tubulin	EF1-α	
CBS110302	*Fusicoccum aesculi*	*Vitis vinifera*	Portugal	AY259092	EU673106	AY573218	Phillips et al., 1997
CMW7780	*F. aesculi*	*Fraxinus excelsior*	South Africa	AY236947	AY236925	AY236896	Slippers et al., 2004
CMW9075	*F. aesculi*	*Populus nigra*	South Africa	AY236950	AY236928	AY236899	Slippers et al., 2004
UCD1333So	*F. aesculi*	*V. vinifera*	California, USA	DQ008327	DQ008350	GU294736	Úrbez-Torres et al., 2006
UCD1156Me	*F. aesuli*	*V. vinifera*	California, USA	DQ233602	DQ233623	GU294734	Úrbez-Torres et al., 2006
CMW9074	*Lasiodiplodia theobromae*	*Pinus* sp.	Mexico	AY236952	AY236930	DQ103565	Slippers et al., 2004
CAA006	*L. theobromae*	*V. vinifera*	California, USA	DQ458891	DQ458859	DQ458876	Alves et al., 2006
UCD191Co	*L. theobromae*	*V. vinifera*	California, USA	DQ008308	DQ008331	EU012397	Úrbez-Torres et al., 2006
UCD205Co	*L. theobromae*	*V. vinifera*	California, USA	DQ008310	DQ008333	EU012398	Úrbez-Torres et al., 2006
CBS115812	*L. gonubiensis*	*Syzygium cordatum*	South Africa	DQ458892	DQ458860	DQ458877	Alves et al., 2006
CMW7775	*Diplodia seriata*	*Ribes* sp.	New York, USA	AY236954	AY236932	AY236903	Slippers et al., 1996
CMW8230	*D. seriata*	n/a	n/a	AY972104	AY972119	DQ280418	de Wet et al., 2005
UCD1035BC	*D. seriata*	*V. vinifera*	Ensenada, Mexico	EU012379	EU012431	EU012402	Úrbez-Torres et al., 2008
UCD1038BC	*D. seriata*	*V. vinifera*	Ensenada, Mexico	EU012380	EU012432	EU012403	Úrbez-Torres et al., 2008
UCD1061BC	*D. seriata*	*V. vinifera*	Ensenada, Mexico	EU012382	EU012434	EU012405	Úrbez-Torres et al., 2008

[a] ITS=Internal transcribed spacer and EF1-α=elongation factor.

[b] Acronyms of cultures collections: CMW = Culture Collection Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CBS = Centraalbureau Schimmelcultures, Utrecht, Netherlands.

Table 3. Morphological descriptions of *Fusicoccum aesculi*, *Lasiodiplodia theobromae* and *Diplodia seriata* isolates from peach trees in Hubei Province, China

Isolates	Conidial size (μm)[a]	Mean ± SD (μm)[b]	Length:width ratio ± SD[b]
Fusicoccum aesculi			
LHKB-222	(19.5-)25.0-32.5 × (5.8-)7.5-7.5	24.8 ± 3.1 × 6.9 ± 0.5	3.6 ± 0.4
XNHG-91WR	(17.0-)22.5-28.0 × (5.0-)7.5-8.5	23.6 ± 2.7 × 6.9 ± 0.8	3.5 ± 0.5
SZ-912	(17.0-)22.5-27.5 × (5.0-)6.3-7.5	22.6 ± 2.5 × 6.0 ± 0.7	3.9 ± 0.6
GA-221	(17.5-)22.5-30.0 × (5.0-)7.5-13.8	22.8 ± 3.1 × 6.9 ± 1.6	3.4 ± 0.7
XC-221	(17.5-)20.0-22.5 × (4.5-)5.0-7.5	20.4 ± 1.2 × 5.4 ± 0.6	3.8 ± 0.4
SZ-422	(16.3-)22.5-27.5 × (4.8-)6.5-7.3	23.3 ± 2.7 × 6.2 ± 0.7	3.8 ± 0.5
Lasiodiplodia theobromae			
JMA-811	(15.0-)23.8-28.0 × (11.0-)14.5-16.0	22.5 ± 1.5 × 14.3 ± 0.9	1.6 ± 0.5
JMB-122	(14.5)-22.5-27.5 × (10.5)-14.0-16.0	22.0 ± 1.4 × 13.9 ± 0.4	1.6 ± 0.3
Diplodia seriata			
GA-312	12.5-25.0-26.8 × 8.5-10.0-11.5	22.5 ± 3.2 × 10.1 ± 0.6	2.3 ± 0.3
GA-422	17.5-22.7-25.0 × 8.8-10.0-11.3	22.7 ± 1.9 × 10.0 ± 0.5	2.2 ± 0.3

[a] Minimum size shown between parentheses followed by medium and maximum size in length and width of 50 conidia recorded from each *Botryosphaeria* isolate from Hubei Province, central China.

[b] SD =standard deviation.

Table 4. Rate of vascular discoloration on detached peach twigs from two to four days after inoculation with a *Fusicoccum aesculi* isolate from Hubei Province, China

Isolate	Vascular discoloration rate (mm/d)	Gum exudation sites[a]
XNHG-241	13.8 ± 1.8 a	n/a
LHKA-222	9.8 ± 5.3 bc	n/a
LHKB-331	9.6 ± 0.5 bc	1
LHKA-221	8.5 ± 3.8 bcd	1
XC-221	8.5 ± 2.6 bcd	1
LHKB-222	8.5 ± 1.8 bcd	1
LHKB-111	8.3 ± 2.1 bcd	3
LHKB-123	8.0 ± 1.2 bcd	n/a
XC-531	7.6 ± 4.8 bcde	n/a
XNHG-252	7.3 ± 7.7 bcde	2
XC-411	6.2 ± 1.8 def	n/a
XNHG-152	5.7 ± 3.8 def	n/a
XC-132	5.3 ± 6.5 cef	1
GA-1113	5.1 ± 4.7 def	4
XNHG-62	3.8 ± 2.3 ef	n/a

[a] Number of gum exudation sites out of 15 inoculated sites for each isolate; n/a = Not available.

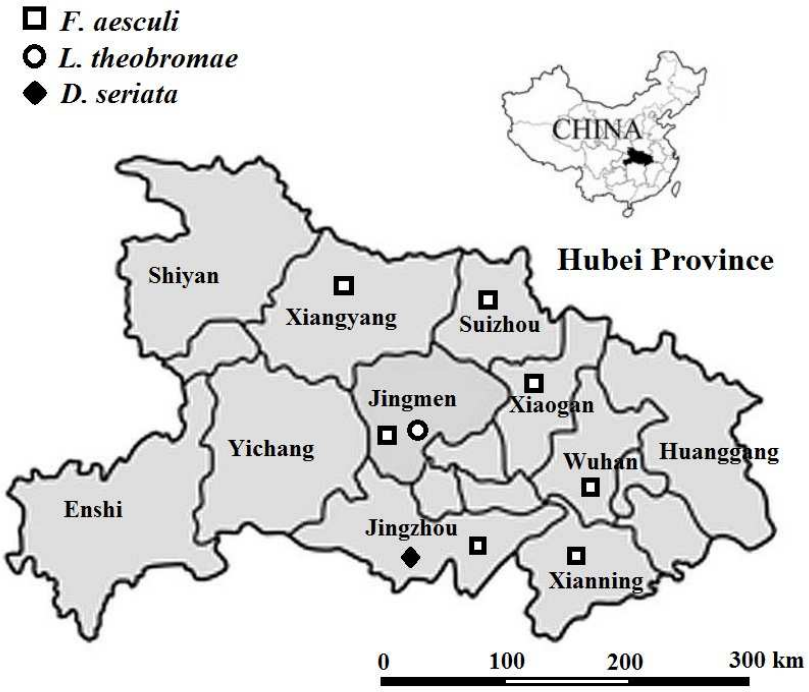

Fig. 1. Geographical distribution of *Botryosphaeria* spp. on peach trees in Hubei Province, central China.

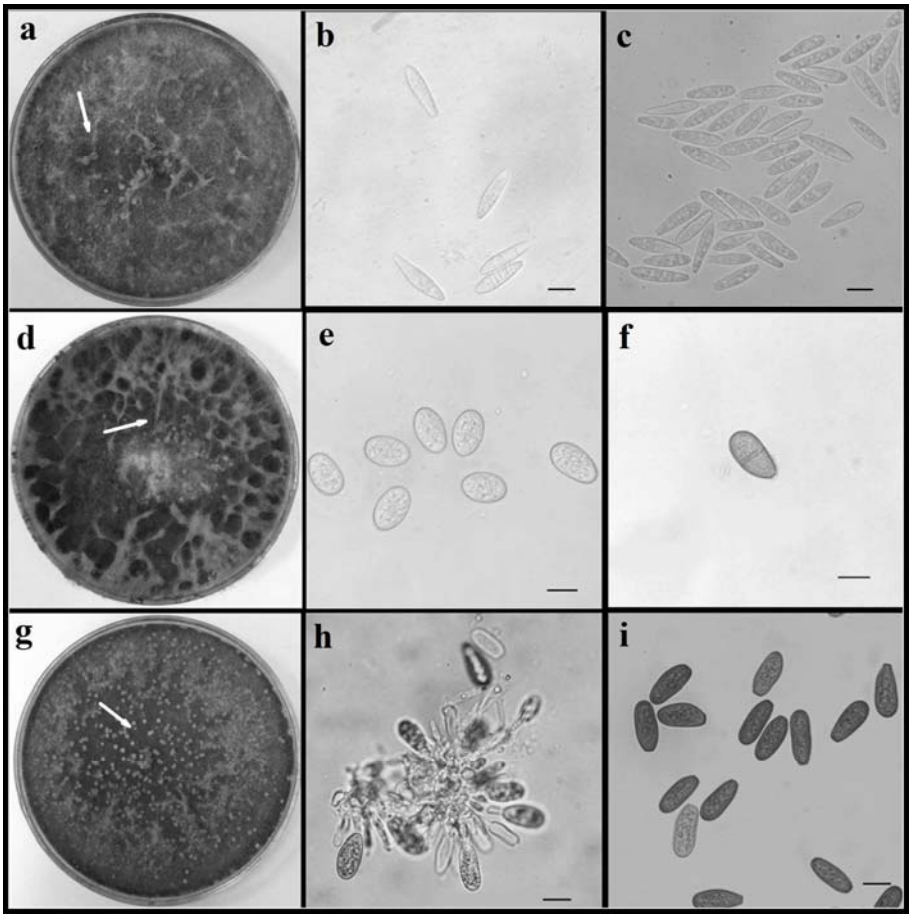

Fig. 2. *Fusicoccum aesculi* (a-c)*, Lasiodiplodia theobromae* (d-f) and *Diplodia seriata* (g-i) conidial morphology. **a**, Colony morphology of 15-day-old *F. aesculi* JMA-121. **b & c**, Hyaline and thin-walled conidia. **d**, Colony morphology of 15-day-old *L. theobromae* (JMB-122). **e**, Young hyaline and thick-walled conidia. **f**, Dark-brown mature conidia. Central septum can be observed. **g**, Colony morphology of 15-day-old *D. seriata* (GA-422). **h**, Conidiogenous cells. **i**, Young hyaline and mature brown conidia. Conidial photographs were taken at ×40 from pycnidia formed in culture. Arrows in a, d and g show the pycnidia in the cultures. Scale bars = 10 μm.

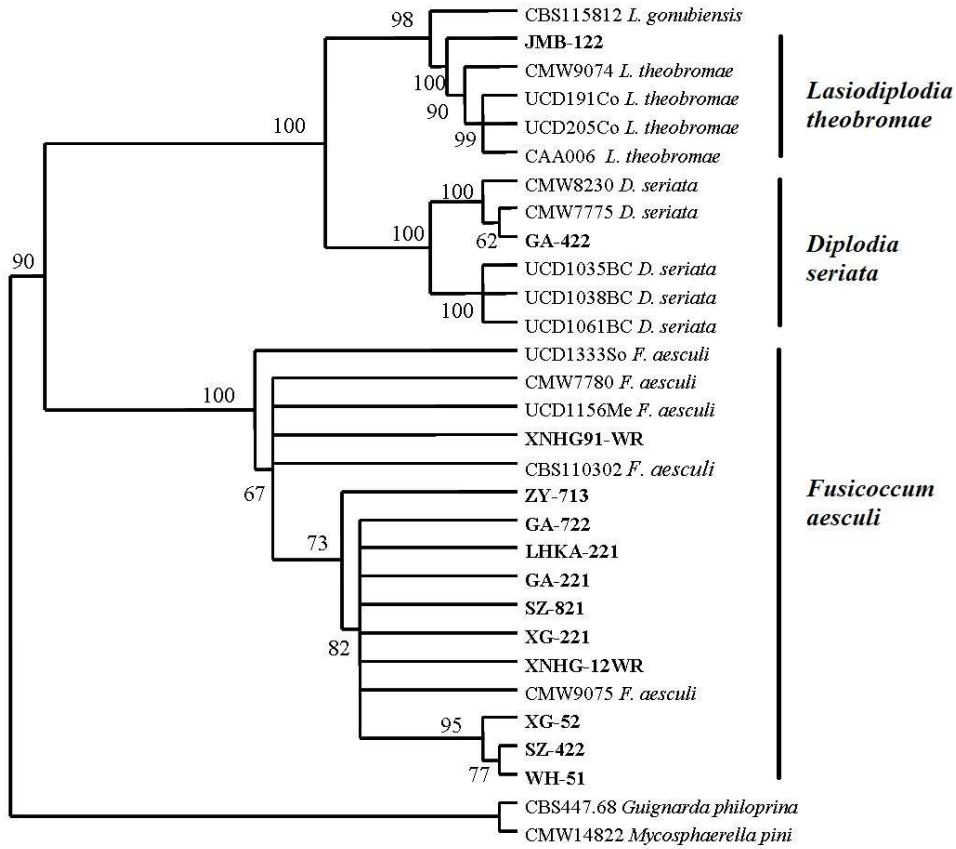

Fig. 3. Most equally parsimonious tree with bootstrap values obtained from the combined internal transcribed spacer, β-tubulin and elongation factor-1 sequence data using 1,000 replicates generated in PAUP* 4.0b10. Isolates from Hubei Province, central China are indicated in bold.