Botanical Studies (2012) 53: 467-478.

New Botryosphaeriaceae fruit rot of mango in Taiwan: identification and pathogenicity

Hui-Fang NI[1,2], Hong-Ren YANG[1], Ruey-Shyang CHEN[3], Ruey-Fen LIOU[2,*], and Ting-Hsuan HUNG[2,*]

[1]*Department of Plant Protection, Chiayi Agricultural Experiment Station, Taiwan Agricultural Research Institute, Chiayi, Taiwan*
[2]*Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan*
[3]*Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan*

(Received February 21, 2012; Accepted May 29, 2012)

ABSTRACT. Mango is an important fruit crop in Taiwan. Recently, severe fruit rot disease was found frequently on harvested mango fruits. To monitor the incidence of disease and to characterize the causal agent, we performed a field survey in the major mango-producing areas of southern Taiwan, including Guntain, Fanshan, and Yujing, during 2009-2011. The results showed a disease incidence ranging from 18.7% to 58.1%, with those of Guntain significantly greater than the incidence found in Yujing and Fanshan. Based on morphological characteristics and nucleotide sequences of the internal transcribed spacer (ITS), β-tubulin gene (TUB) and elongation factor 1-alpha (EF1-α) gene, we identified four Botryosphaeriaceae species, including *Fusicoccum aesculi*, *Neofusicoccum mangiferae*, *N. parvum*, and *Lasiodiplodia theobromae*. Pathogenicity tests indicated that all of these fungal species were pathogenic to harvested mango fruits, and *L. theobromae* was the most aggressive pathogen. Moreover, when attached, immature mango fruits were inoculated with conidia of Botryosphaeriaceae species, disease symptoms characteristic of fruit rot appeared on the fruits after harvest and ripening. These findings indicated that *L. theobromae, F. aesculi*, *N. mangiferae*, and *N. parvum* were all causal agents of the new fruit rot of mango. Furthermore, their conidia may serve as important sources of inocula causing fruit rot disease in mango orchards.

Keywords: Botryosphaeriaceae; Fruit rot; Mango.

INTRODUCTION

Mango (*Mangifera indica*) is an economically important fruit crop in Taiwan. According to Agricultural Statistics Yearbook 2010 (http://www.coa.gov.tw/view.php?catid=23771), the total area of mango cultivation in Taiwan was 16,796 ha, which led to the production of 135,293 metric tons of mango fruits, with a yearly value over 157 million US dollars. The main area for mango cultivation is located in the southern part of Taiwan, including Tainan, Kaohsiung, and Pingtung.

Postharvest diseases, which cause serious problems during storage and transportation of mango fruits, are the major factors that limit the thriving mango industry. Both anthracnose disease caused by *Colletotrichum gloeosporioides* (Ploetz, 1994; Yang and Leu, 1988; Arauz, 2000) and stem-end rot caused by *Lasiodiplodia theobromae* (Liao, 1975; Johnson and Cooke, 1991; Ploetz, 1994) or *Phomopsis mangiferae* (Ploetz, 1994; Ko et al., 2009) are usually considered to be the most severe postharvest disease of mango worldwide. However, we have recently found that many harvested mango fruits displayed brown soft lesions on the body surface of ripe mango fruit, rather than at the pedicel end, which indicated that fruit rot could be another serious postharvest disease in Taiwan.

Botryosphaeriaceae species are known to occur worldwide, causing dieback, cankers, shoot blights, leaf spot, gummosis, and fruit rots in a wide range of plant hosts which play important roles in agriculture and forestry (Phillips, 2002; van Niekerk et al., 2004; Slippers et al., 2005; Damn et al., 2007; de Macedo and Barreto, 2008; Marincowitz et al., 2008; Javier-Alva et al., 2009; Yu et al., 2009; Wang et al., 2011).

In Taiwan, the first plant pathogenic species of Botryosphaeriaceae that caused mango stem-end rot was reported by Liao (1975). He isolated the pathogen *Diplodia natalensis* (syns. *Lasiodiplodia theobromae* and *Botryodiplodia theobromae* (teleomorph: *Botryosphaeria rhodina*)) and confirmed its pathogenicity. Recent studies from several laboratories demonstrated that a complex of Botryosphaeriaceae pathogens (Slippers et al., 2005; de Oliveira

*Corresponding authors: E-mail: rfliou@ntu.edu.tw; Fax: 886-2-23620271; Tel: 886-2-33665208 (Ruey-Fen LIOU); E-mail: thhung@ntu.edu.tw; Fax: 886-2-23636490; Tel: 886-2-33664600 (Ting-Hsuan HUNG).

Costa et al., 2010), including *L. theobromae*, *N. mangiferae*, *Neofusicoccum parvum* and *Fusicoccum aesculi*, are associated with stem-end rot of mango. Take for example Australia, where *F. aesculi* (= *B. dothidea*), *N. mangiferae, N. parvum, L. theobromae*, and *Fusicoccum* sp. cause stem-end rot of mango (Slippers et al., 2005). In Brazil, *L. theobromae*, *F. aesculi*, and *N. parvum* have been reported as pathogens of mango stem-end rot and dieback disease (de Oliveira Costa et al., 2010).

Differentiation of Botryosphaeriaceae species were in the past chiefly based on the morphology of their anamorphs (Jacobs and Rehner, 1998; Denman et al., 2000). However, morphological characteristics of these fungi may vary within the species, and in some cases, they may look very similar between species, making identification of the fungus even more difficult. For efficient identification, DNA-based techniques have been applied to the taxonomy of Botryosphaeriaceae (Denman et al., 2000; Slippers et al., 2004; Alves et al., 2005; Taylor et al., 2005; Crous and Groenewald, 2005; Crous et al., 2006; De Wet et al., 2008). Combination of the molecular techniques with morphological characteristics has been used to successfully identify *F. aesculi*, *N. parvum*, and *N. ribis*, all of which were previously classified as *F. aesculi* (= *B. dothidea*) (*sensu* von Arx and Müller, 1954) (Jacobs and Rehner, 1998; Smith and Stanosz, 2001; Slippers et al., 2004; Crous et al., 2006).

Currently, fungal pathogens known to cause mango fruit rot include only *Alternaria alternata*, *Phytophthora nicotianae*, *Pestalotiopsis mangiferae*, and *Phyllosticta anacardiacearum*, according to "Common Names of Plant Diseases" posted on the website of the American Phytopathological Society (http://www.apsnet.org/publications/commonnames/Pages/Mango.aspx). Information regarding the role of Botryosphaeriaceae species on mango fruit rot has been very limited. The aims of this study were to: a) identify Botryosphaeriaceae isolates collected from fruit rot of mango fruits, b) investigate the incidence of fruit rot and frequency of Botryosphaeriaceae species in three major mango producing areas of Taiwan, including Fanshan (Kaohsiung), Yujing, and Guntain (both in Tainan) during 2009-2011; c) test the pathogenicity and compare the virulence of Botryosphaeriaceae isolates obtained from mango fruits with fruit rot.

MATERIALS AND METHODS

Field survey, disease symptoms, and fungal isolation

Field surveys were conducted at 77 orchards located in the Pintung (Fanshan) and Tainan (Yujing and Guntain) areas of Taiwan during 2009-2011, and 15-20 mango fruits were randomly collected from each orchard. The incidences of fruit rot disease were calculated 7 days after harvest according to the following formula: Disease incidence (%) = (Number of fruits which showed only fruit rot but not anthracnose symptoms/ Total number of fruits) × 100%. Fruits with typical symptoms were then selected for fungal isolation. The epidermis of fruit was first disinfested with 70% (v/v) ethanol and air-dried. Subsequently, small pieces (2-3 mm^2) of necrotic tissue were dissected from the margins of lesions on fruit and placed on an acidified (750 μL of a 50% (v/v) solution of lactic acid per 300 mL of potato dextrose agar medium) (APDA) (Merck KGaA, Darmstadt, Germany). The plates were incubated at room temperature for 1-2 weeks. Putative Botryosphaeriaceae species isolates, recognized by their rapidly growing colonies with gray mycelium (Lazzizera et al., 2008), were subcultured on potato-dextrose agar (PDA) plates. Isolates were stored on PDA slants at 8°C. The frequency of occurrence of the fungi in the collected fruits, which showed characteristic symptoms of fruit rot, was calculated according to the following formula: Frequency of occurrence (%) = (Number of fruits colonized by a specific pathogen/ Total number of fruits with fruit rot symptoms) x 100%.

Morphological characterization

For studies on colony morphology, isolates were grown on PDA and incubated at 25°C in darkness. The morphology of mycelium and conidia (dimensions, shape, color, presence of septa and longitudinal striations) were recorded. To induce sporulation, putative Botryosphaeriaceae isolates were grown on 2% water agar plates containing sterilized pine (*Pinus morrisonicola*) needles (Smith et al., 1996), and incubated at 25°C with a 12-h light (near UV)/ dark cycle. For further purification, conidia released from pycnidia on the pine needles were spread on water agar. After 12-24 h, single germinating conidia were picked and transferred to PDA. Morphology of the conidia formed on pine needles was also examined under a stereoscopic microscope (Nikon SMZ 1500, Tokyo, Japan). To determine the average length and width of conidia, at least 50 conidia from each isolate were analyzed using a light microscope (Nikon, ECLIPSE 80i, Tokyo, Japan); their images were photographed with a pixel camera system (Pixera Penguin 600CL, Los Gatos, CA, USA) and the length and width of conidia were measured by using a Simple PCI software Rev. 3.6 (Compix Inc., Cranberry Township, PA, USA). The measurements of conidia were subjected to statistical analyses and presented as average ± standard deviations.

Molecular characterization

The genomic DNA of fungal mycelia was isolated using the method described by Wang et al. (1993). For comparative phylogenetic study, partial sequences of three housekeeping genes were amplified by PCR, including ribosomal internal transcribed spacer (ITS), β-tubulin (TUB), and elongation factor 1-α (EF1-α). The PCR mixture contained 1X PCR buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% (w/v) gelatin, 1% Triton X-100), 100 μM of each dNTP, 0.2 μM of each primer, 0.4 U of Prozyme DNA polymerase (Protech Technology Enterprise, Taipei, Taiwan). Primers used for the amplification of each gene were: ITS1 (5'-TCC GTA GGT

GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al., 1990; Wang et al., 2010) for ITS, Bt2a (5'-GGT AAC CAA ATC GGT GCT GCT TTC-3') and Bt2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3') for TUB (Glass and Donaldson, 1995), EF1-728F (5'-CAT CGA GAA GTT CGA GAA GG-3') and EF1-986R (5'-TAC TTG AAG GAA CCC TTA CC-3') for EF1-α (Carbone and Kohn, 1999), respectively. The amplification program included an initial step of 4 min at 94°C, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and elongation at 72°C for 30 sec. A final extension was performed at 72°C for 10 min. The reaction was carried out by using a Bio-Rad iCycler Thermal cycler (Hercules, California, USA). The amplified products were sequenced in both directions by the dideoxy termination method through a service provided by the Tri-I Biotech (Taipei, Taiwan).

For phylogenetic analysis, multiple sequence alignment was performed with nucleic acid sequences of ITS rDNA, TUB, and EF1-α obtained from this study as well as those retrieved from GenBank (Table S1), by using the CLUSTAL X (Thompson et al., 1997), and a phylogram was generated by the application of neighbour-joining (NJ) algorithm in PAUP 4.0 beta 10 (Sinauer Associates, Sunderland, MA, USA). Bootstrap analysis was performed with 1000 replicates to test the branch strength.

Pathogenicity test

Pathogenicity of Botryosphaeriaceae species isolated from diseased fruits was assessed by the use of either attached or harvested mango fruits. For inoculation of attached fruits in the orchard, 2 μL of conidial suspension (100 spores), which was prepared as described before, was dropped on the surface of an attached, immature mango fruit (cv. Irwin), followed by the addition of 20 μL water agar to immobilize the conidia. The inoculated fruit was then put inside a paper bag for protection. Each fungal isolate was inoculated on twenty fruits. Four to 6 weeks post inoculation, the fruits were harvested and treated with calcium carbide to accelerate ripening. Incidence of mango fruit rot was recorded 2 weeks after harvest.

For inoculation of the harvested fruits, a total of nine isolates representing four different species of Botryosphaeriaceae were selected for pathogenicity tests. For inoculation, fungal isolates were grown on PDA at 25°C for 7 days. Unripe but mature mango fruits (cv. Irwin) were collected from Yujing (Tainan, Taiwan) and treated with hot water (60°C) for 20 sec to avoid possible interference from latent infections of postharvest pathogens. Prior to inoculation, fruits were pricked with a sterile needle, and small discs of agar (5 mm in diameter) colonized by Botryosphaeriaceae isolates were placed on the wound. Each fungal isolate was inoculated on three fruits. As a control, a parallel experiment was performed by covering the wounded zone with discs of PDA without Botryosphaeriaceae. Pathogenicity of the fungal isolates was determined based on the length of lesions (mm) that developed 7 days after inoculation. Statistical analyses of the data were performed by using SAS (version 8, SAS Institute) with the Fisher's protected test, and an F value with $P<0.05$ was considered significant.

RESULTS

Field survey of mango fruit rot disease and collection of fungal isolates

To survey the occurrence of mango fruit rot disease, we collected mango fruits from three of the major mango producing areas in Southern Taiwan, including Fanshan (650 fruits from 32 orchards), Yujing (440 fruits from 22 orchards), and Guntain (454 fruits from 23 orchards). We examined all the fruits for the appearance of symptoms and found a total of 490 mango fruits with characteristic symptoms of fruit rot, which appeared as browning, soft, and watery lesions, different from that of anthracnose. The incidence of fruit rot disease identified in Guntain (46.0% in 2009, 53.8% in 2010, and 58.1% in 2011) was significantly greater than those found in Yujing (35.0% in 2009, 25.6% in 2010, and 23.8% in 2011) and Fanshan (20.1% in 2009, 18.7% in 2010, and 22.5% in 2011) (Table 1). The results indicated that this disease occurred frequently in all three mango producing areas.

Isolates and morphological characterization

From the mango fruits showing symptoms of fruit rot (Figure 1), we obtained 237 isolates of Botryosphaeriaceae species. Based on their colony morphology on APDA plates, these Botryosphaeriaceae isolates were classified into four groups, and 5 to 10 isolates were randomly selected from each group for further studies.

When cultured on PDA or water agar containing pine needles, most isolates sporulated within 21 days of incubation. No teleomorph was observed for any isolate during this study. Based on the morphology of colonies and conidia, all Botryosphaeriaceae isolates were classified as one of four distinct species, including *L. theobromae*, *N. mangiferae*, *N. parvum* and *F. aesculi* (Table 2).

When subcultured on PDA, *L. theobromae* initially produced white, fluffy aerial mycelium that rapidly covered the surface of Petri dishes within two days of incubation. The mycelium then turned to pale olivaceous gray within 3-4 days, and produced pycnidia after 7 days. When visualized from the bottom of the Petri dish, the colonies first showed white to olivaceous gray, and became dark olivaceous after 7-10 days. It produced pycnidia while incubated in water agar (WA) supplemented with sterilized pine needles after 7-14 days. Immature conidia were hyaline, aseptate, with the shape of ellipsoid to ovoid. They became uniseptate, thick-walled, light brown pigmented with longitudinal striations once mature (Figure 1A). The average length (L) and width (W) of 400 conidia was 23.40-27.18 x 12.47-15.08 μm, with an L/W ratio of 1.61-1.98 (Table S2).

Table 1. The incidence of fruit rot disease and occurrence frequency of Botryosphaeriaceae species on mango fruits exhibiting symptoms of fruit rot during 2009-2011.

Region	Year	Incidence (%)[a]	Frequency of occurrence (%)[b]			
			Lasiodiplodia theobromae	*Neofusicoccum parvum*	*Neofusicoccum mangiferae*	*Fusicoccum aesculi*
Fanshan	2009	20.1	1.8	16.1	28.6	12.5
	2010	18.7	6.4	25.5	4.3	34.0
	2011	22.5	0.0	37.8	20.0	37.8
Yujing	2009	35.0	9.7	3.2	3.2	25.8
	2010	25.6	2.8	30.6	0.0	47.2
	2011	23.8	10.5	26.3	7.9	13.2
Guntain	2009	46.0	7.5	0.0	2.5	17.5
	2010	53.8	3.3	5.0	0.0	8.3
	2011	58.1	13.1	3.6	2.2	12.4

[a]Incidence of mango fruit rot in each area was calculated by the following formula: Disease incidence (%) = (Number of fruits which showed only symptoms of fruit rot but not anthracnose/Total number of fruits) × 100%.
[b]Frequency of occurrence (%) = (Number of fruits colonized by a pathogen/Total number of fruits with fruit rot symptoms) × 100%.

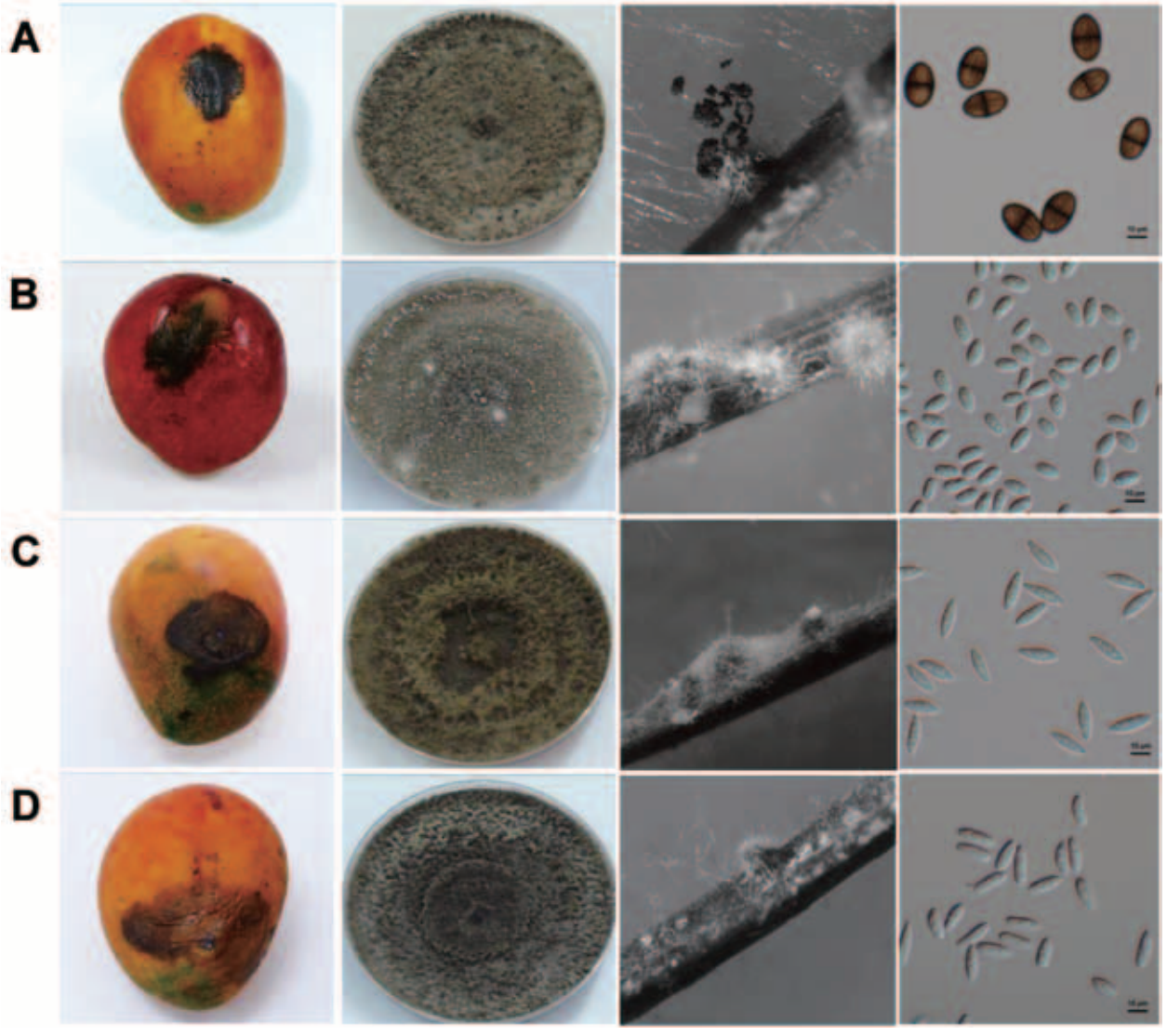

Figure 1. Symptoms of mango fruit rot and morphology of fungal colony, pycnidia, and conidia. A: *Lasiodiplodia theobromae*; B: *Neofusicoccum mangiferae*; C: *Fusicoccum aesculi*; D: *N. parvum*. Bar = 10 μm.

Table 2. Representative isolates of Botryosphaeriaceae species collected from mango fruits with fruit rot in southern Taiwan.

Isolate	Identity	Locality	GenBank Accession No.		
			ITS	EF1-α	TUB
B961	*Lasiodiplodia theobromae*	Guntain	GQ502453	GQ979999	GU056845
B965	*L. theobromae*	Guntain	GQ502454	GQ980000	GU056854
B838	*L. theobromae*	Fangshan	GQ502456	GQ980001	GU056852
B852	*L. theobromae*	Guntain	GQ502457	GQ980002	GU056851
B918	*L. theobromae*	Guntain	GQ502458	GQ980003	GU056850
B902	*L. theobromae*	Guntain	GQ502459	GQ980004	GU056849
B878	*L. theobromae*	Guntain	GQ502460		GU056848
B607	*L. theobromae*	Guntain	GQ502461		GU056846
B845	*Neofusicoccum parvum*	Fangshan	GQ861434	GQ985316	GU062771
B946	*N. parvum*	Yujing	GQ861432	GQ985313	GU062768
B1314	*N. parvum*	Fangshan	GU073291	GU121436	GU111537
B794	*N. parvum*	Fangshan	GQ861433	GQ985312	GU062767
B1260	*N. parvum*	Fangshan	GU073287	GU121432	GU111533
B1272	*N. parvum*	Yujing	GU073288	GU121433	GU111534
B1296	*N. parvum*	Yujing	GU073289	GU121434	GU111535
B1307	*N. parvum*	Fangshan	GU073290	GU121435	GU111536
B809	*N. mangiferae*	Fangshan	GQ848323	GQ998898	GU071122
B793	*N. mangiferae*	Fangshan	GQ848320	GQ998900	GU071120
B808	*N. mangiferae*	Fangshan	GQ848322	GQ998899	GU071121
B979	*N. mangiferae*	Yujing	GQ848315	GQ998897	GU071123
B763	*N. mangiferae*	Fangshan	GQ421486	GQ998896	GU071119
B964	*Fusicoccum aesculi*	Guntain	GQ861429	GU002157	GU071124
B811	*F. aesculi*	Fangshan	GU453689	GU002164	GU071125
B844	*F. aesculi*	Fangshan	GU453690	GU002163	GU071126
B922	*F. aesculi*	Yujing	GQ421485	GU002162	GU071127
B833	*F. aesculi*	Fangshan	GQ861430	GU002161	GU071129
B801	*F. aesculi*	Fangshan	GQ861431	GU002160	GU071130
B932	*F. aesculi*	Yujing	GQ861428	GU002159	GU071131
B1113	*F. aesculi*	Yujing	GU453691	GU002158	GU071132

Isolates of *N. mangiferae* initially produced white, appressed mycelium. Four days after incubation on PDA, the middle of colony turned to pale olivaceous gray and produced pycnidia 10-14 days after incubation. When visualized from the bottom of Petri dish, the colonies were olivaceous to black. This fungus grew slower than the other three *Botryosphaeria* species found in this study. Pycnidia were formed on WA supplemented with sterilized pine needles after 3-7 days. The conidia were hyaline and ovoid (Figure 1B), with an average length and width of 11.98-12.93 × 6.25-6.98 μm (L/W= 1.85-1.95) (Table S2).

Colonies of *F. aesculi* were initially white with aerial mycelium. They became pale olivaceous gray from the center of colony after 3-4 days, and turned black after 7 days. Pycnidia were formed on WA supplemented with sterilized pine needles after 1-2 weeks. Conidia were hyaline, thin-walled, aseptate, and fusiform (Figure 1C), with an average length and width of 18.72-22.10 × 5.72-6.63 μm (L/W= 3.05-3.52) (Table S2).

Cultures of *N. parvum* were initially white with aerial mycelium. The middle of colony then became pale olivaceous gray after 3-4 days. When visualized from the bottom of Petri dish, the colony was deep olivaceous gray to black after 4-7 days of incubation. Pycnidia were formed on WA supplemented with sterilized pine needles for 2-3 weeks. Conidia were hyaline, aseptate, and fusiform (Figure 1D). The average length and width of 416 conidia were 15.85-19.25 × 4.49-6.61 μm (L/W= 2.70-3.68) (Table S2).

Phylogenetic analysis

To confirm the morphometric identifications and to infer the evolutionary relatedness among these Botryosphaeriaceae species, partial nucleotide sequences of ITS, TUB, and EF1-α from *L. theobromae*, *N. mangiferae*, *N. parvum*, and *F. aesculi* were amplified by PCR. The respective length of amplified products of ITS, TUB, and EF1-α for *L. theobromae* were approximately 540, 460, and 320 bp, respectively, while those for *N. mangiferae, N. parvum*, and *F. aesculi* were 580, 460, and 300 bp, respectively. Sequences of these amplified products were compared with those deposited in GenBank (Table 2), and performed multiple sequence alignments to detect differences among these species. Sequences of the ITS, TUB, and EF1-α genes from isolates of the same species showed an identity ranging from 98% to 100%. In contrast, nucleotide sequence of ITS, TUB, and EF1-α showed a respective identity range of 93-98%, 91-98%, and 75-96% among different species.

In addition, three unrooted phylogenetic trees were constructed based on multiple sequence alignment of ITS, TUB, and EF1-α. The overall topology of these trees was similar, with each tree composed of four major clades (Figure 2). Isolates from the same species formed a single, monophyletic group with a bootstrap support ranging from 90% to 100%. Moreover, the clade representing *N. mangiferae* is close to that of *N. parvum* in all three trees.

Frequency of Botryosphaeriaceae species in southern Taiwan

To know the frequency of occurrence of each fungal pathogen in different mango producing areas, the per-

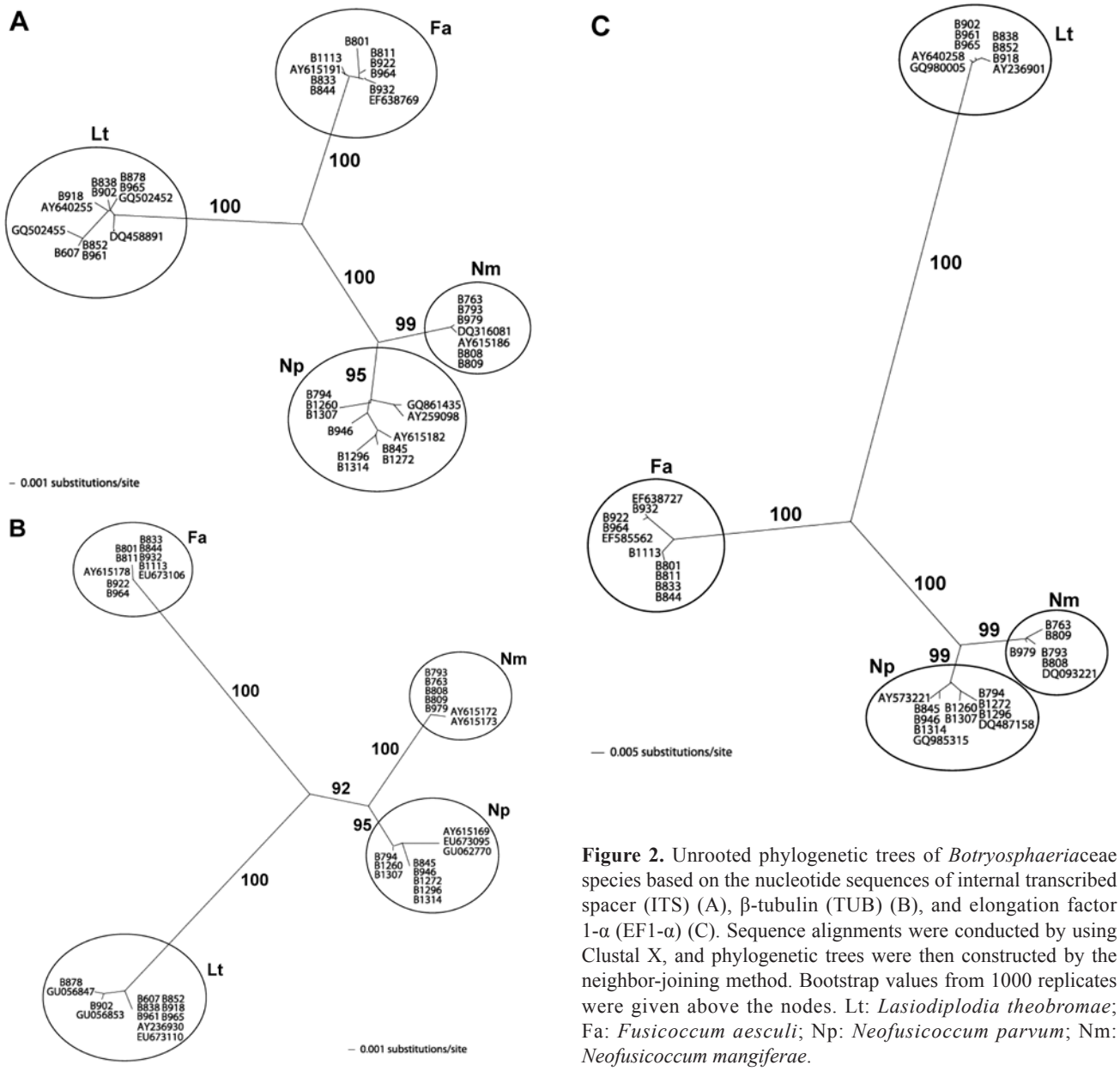

Figure 2. Unrooted phylogenetic trees of *Botryosphaeria*ceae species based on the nucleotide sequences of internal transcribed spacer (ITS) (A), β-tubulin (TUB) (B), and elongation factor 1-α (EF1-α) (C). Sequence alignments were conducted by using Clustal X, and phylogenetic trees were then constructed by the neighbor-joining method. Bootstrap values from 1000 replicates were given above the nodes. Lt: *Lasiodiplodia theobromae*; Fa: *Fusicoccum aesculi*; Np: *Neofusicoccum parvum*; Nm: *Neofusicoccum mangiferae*.

centage of fungal isolates collected from the diseased mango fruits were calculated. As shown in Table 1, at least three Botryosphaeriaceae species were found in Fanshan, Yujing, and Guntain, respectively, but their relative prevalence was different. In 2009, the major pathogen in Fanshan was *N. mangiferae*, while that for Yujing and Guntain was *F. aesculi*. In 2010, *F. aesculi* and *N. parvum* were predominant in Fanshan and Yujing. They were also found in Guntain, but only with a low frequency. In 2011, both *F. aesculi* and *N. parvum* were found frequently in Fanshan, while only *N. parvum* was predominant in Yujing. In Guntain, both *L. theobromae* and *F. aesculi* occurred more frequently than *N. mangiferae* and *N. parvum*. It was obvious that, even in the same region, incidence of mango fruit rot caused by some pathogens differed greatly between years. For example, the occurrence frequency of *N. mangiferae* in Fanshan varied from 28.6% in 2009 to 4.3% in 2010, and then to 20.0% in 2011. Moreover, the frequency of *N. mangiferae* in Yujing and Guntain during 2009-2011 was less than 8% and 3% of the total rotted mangos, respectively.

Pathogenicity tests

To evaluate the pathogenicity of the Botryosphaeriaceae isolates, inoculation experiments on attached, immature mango fruits was performed with conidial suspensions of *L. theobromae*, *N. mangiferae*, *N. parvum* or *F. aesculi*. Four to 6 weeks after inoculation, the inoculated fruits were harvested and ripened by a treatment using calcium carbide. Fruit rot symptoms appeared on the fruit body as fruits ripened gradually, no matter which pathogen was used as the inoculum. When examined 2 weeks after harvest, most of the inoculated fruits showed symptoms characteristic of fruit rot (Table 3). In contrast, the mock-inoculated fruits appeared healthy and intact. To make sure that lesions on the diseased fruits were caused by Botryosphaeriaceae species, the pathogens were reisolated from the lesion edge and examined as described in the previous section. These results indicated that symptoms development on inoculated fruit was indeed caused by the specific Botryosphaeriaceae isolate originally used as the inoculum.

Table 3. Incidence of fruit rot on mature mango fruits after inoculation of attached mango fruits with *L. theobromae*, *N. mangiferae*, *F. aesculi*, or *N. parvum*.[a]

Pathogen	No. diseased/ No. harvested fruits[b]	
	Exp. 1	Exp. 2
L. theobromae	18/20	17/20
N. mangiferae	20/20	12/15
F. aesculi	15/17	15/19
N. parvum	17/20	18/20
Control	0/20	0/15

[a]Conidia of the fungal pathogen were inoculated on the immature fruits which were still attached on the mango trees.
[b]Four to 6 weeks post inoculation, the fruits were harvested and ripened by the treatment with calcium carbide. Incidence of fruit rot was recorded 2 weeks after harvest of the mango fruits.

Table 4. Mean length of lesions caused by the isolates of Botryosphaeriaceae species following inoculation on wounded or unwounded mango fruits (cultivars 'Irwin') for 7 days.

Isolate	Mean lesion length (mm)[a] (wounded)	Mean lesion length (mm) (unwounded)
Neofusicoccum mangiferae (B763)	43.4 bc	18.9 b
Neofusicoccum mangiferae (B793)	42.3 bc	9.7 b
Fusicoccum aesculi (B811)	41.4 c	11.3 b
Fusicoccum aesculi (B932)	45.5 bc	8.0 b
Lasiodiplodia theobromae (B826)	101.9 a	56.7 a
Lasiodiplodia theobromae (B878)	114.4 a	73.3 a
Neofusicoccum parvum (B837)	66.5 b	55.3 a
Neofusicoccum parvum (B1001)	60.8 bc	26.0 b
Neofusicoccum parvum (B010)	60.4 bc	29.3 b
LSD ($P = 0.05$)	25.0	25.4

[a]Means followed by the same letter are not significantly different.

Furthermore, inoculation experiments were also performed with harvested mango fruits. When inoculated on the harvested fruits, all Botryosphaeriaceae isolates resulted in the formation of black-brown lesions of irregular shape on the surface of both wounded and unwounded fruits within 7 days post inoculation. In contrast, no lesion developed on wounded or unwounded fruits of the control experiment. Recovery of fungal isolates from the lesion edge of the diseased fruits confirmed that the fruit rot symptoms was indeed caused by a Botryosphaeriaceae isolate which was used as inoculum. Measurement of lesion lengths on the inoculated mango fruits followed by statistical analyses indicated that the size of lesions caused by *F. aesculi*, *N. mangiferae*, and *N. parvum* showed no significant difference on both wounded and unwounded fruits (Table 4). In contrast, the lesion size caused by *L. theobromae* was significantly larger than those of the other three pathogens. Nonetheless, when the inoculation experiment was performed on unwounded fruits, isolates of the same Botryosphaeriaceae species differed in virulence as seen in the case of *N. parvum*. The size of lesions caused by isolate B837 on unwounded fruits (55.3 mm) was significantly larger than those caused by the other two *N. parvum* isolates, B1001 and B010, but showed no significant difference from those of *L. theobromae*. It is also worth mentioning that, when the inoculation was performed with wounded fruits, the lesions caused by all four pathogens developed faster and ended up with the formation of lesions with a bigger size compared to those observed on unwounded fruits (Table 4).

DISCUSSION

Mango fruit rot is characterized by the appearance of brown to dark spots on the epidermis of the fruit body. Subsequently, the affected areas will rapidly split open and become soft and watery. Therefore, once infected, mango fruits completely lose their commercial values. As revealed by its high incidence, fruit rot has become an important post-harvest disease of mango in Taiwan. However, little is known about pathogens causing this disease. Based essentially on characteristics of the anamorph, such as morphology of fungal colonies and conidia, we have identified four pathogens which belong to Botryosphaeriaceae, including *L. theobromae*, *N. mangiferae*, *N. parvum*, and *F. aesculi*. Nonetheless, *F. aesculi* and *N. parvum* remained difficult to identify, because of their similarity in colony morphology as well as size and shape of conidia. Indeed, these two species have in former years been treated as part of the *B. dothidea* complex (Smith and Stanosz, 2001; Crous et al., 2006). In this study, phylogenetic analysis of the sequences of ITS, TUB, and EF1-α clearly separated *N. parvum* from *F. aesculi*, indicating that molecular characteristics were useful for the differentiation of Botryosphaeriaceae species. In support of our results, *L. theobromae*, *F. aesculi*, *N. mangiferae*, and *N. parvum* are known to form distinct clades by phylogenetic analysis (Slippers et al., 2005; Damm et al., 2007; de Oliverira Costa et al., 2010).

In culture, isolates of *L. theobromae* grew much faster than the other three fungal species, able to fully colonize a 90-mm Petri dish within 48 h. Furthermore, they produced conidia with pigment and longitudinal striations, which were quite different from those of the other species. As an important pathogen of woody hosts, *L. theobromae* has been reported to cause cankers, dieback, fruit rot, and root rots on over 500 different hosts, including perennial fruits, nut trees, vegetable crops, and ornamental plants (Punithalingam, 1980; Alves et al., 2008; Úrbez-Torres et al., 2008; Abdollahzadeh et al., 2010). In Taiwan, *L. theobromae* is also known to cause stem canker and fruit rot of guava as well as stem-end rot of mango and papaya (Wang and Hsieh, 2006; Wang et al., 2007). In the present study, we verified that *L. theobromae* is one of the fungal pathogens that causes mango fruit rot. Moreover, as revealed by the inoculation experiment, *L. theobromae* was more virulent than the other three Botryosphaeriaceae pathogens, which was also noticed by de Oliverira-Costa et al. (2010). It is also interesting to notice that, despite *L. theobromae* being highly virulent, incidence of fruit rot caused by this pathogen was relatively low in Fanshan, Yujing, and Guntain, compared to those caused by the other three pathogens. It is likely that the inoculum density of *L. theobromae* was low in these areas. Further investigation will be conducted to investigate the relationship between the inoculum density and disease incidence.

Neofusicoccum parvum and *F. aesculi*, both are first reported as pathogens of mango in Taiwan, were the dominant species associated with mango fruit rot during this survey. *N. parvum* is known to be associated with stem-end rot of mango in Australia (Slippers et al., 2005) and Brazil (de Oliveira-Costa et al., 2010), and dieback of mango in Peru (Javier-Alva et al., 2009). In addition, it has been reported as the pathogen of stem canker and dieback of Asian pear trees in Taiwan (Shen et al., 2010). On the other hand, *F. aesculi* is known to cause canker and fruit rot in numerous woody plants, such as fruit rot of olives (Phillips et al., 2005), canker in grapevines (Úrbez-Torres et al., 2006), shoot and panicle blight in eucalyptus (Yu et al., 2009), stem-end rot of mango in Brazil (de Oliveira Costa et al., 2010) and Australia (Slippers et al., 2005). In Taiwan, *F. aesculi* caused the ring rot of Pyrus and stem canker of Salix (Tsai et al., 1991). In this study, we found that, in 2010 and 2011, *N. parvum* and *F. aesculi* were the major Botryosphaeriaceae species that caused mango fruit rot in Fanshan and Yujing. In 2009, the dominant pathogen in Fanshan was *N. mangiferae*, while that in Yujing was *F. aesculi*. *N. mangiferae* has been found as the pathogen of stem-end rot of mango (Slippers et al., 2005), and the casual agent of avocado fruit rot in Taiwan (Ni et al., 2009). The reason determining which fungus is the dominant pathogen for causing mango fruit rot can be complicated, because factors such as survival of fungus in the field, inoculum source, inoculum density, climate, and cultivation practices all could affect the dynamics of pathogens as well as the frequency of disease incidence in the field (Niekerk et al., 2011; Sakalidis et al., 2011).

Furthermore, it is also interesting to note that the total frequency of Botryosphaeriaceae species in Guntain was lower than that in Fanshan and Yujing, despite of the high incidence of fruit rot in this area. Indeed, *Phomopsis* spp. were isolated at a high rate in Guntain, suggesting the roles of additional fungal pathogens other than Botryosphaeriaceae species as causal agents of mango fruit rot (unpublished data).

Although the causal agents of both mango fruit rot and stem-end rot are *L. theobromae*, *F. aesculi*, *N. mangiferae*, and *N. parvum*, fruit rot is a disease distinct from stem-end rot. Johnson and Cooke (1991) suggested that these pathogens may occur as endophytes in mango stem tissue and colonize the stem end of mango fruits during maturation, thereby causing stem-end rot. In this study, however, lesions caused by Botryosphaeriaceae species were usually found on the fruit body, suggestive of an infection pathway different from that of stem-end rot. Inoculum may come from conidial ooze generated from dead twigs of mango trees (unpublished data), or fungal spores in soil and leaf litter around mango orchards (Johnson, 2008). In support of this idea, as shown in the inoculation experiments performed with attached, immature mango fruits, conidia of these Botryosphaeriaceae species were able to infect the fruits successfully. Therefore, to reduce the incidence of fruit rot, it is important to remove the dead twigs and branches for routine maintenance of orchard hygiene, and also to wrap developing fruits inside paper bags for protection against conidia residing in soil and diseased plant

tissues on the ground. When the inoculation experiments were performed with wounded mango fruits, the symptoms developed faster than that on unwounded fruits. It is likely that fruit sap released by the wounds may serve as nutrients of the mycelium, and thus lead to rapid growth of the fungus onto the plant tissue (Amponsah et al., 2011). It is thus also important to avoid the making of any wound on the mango fruits to reduce the incidence of fruit rot.

The present work is the first comprehensive study of Botryosphaeriaceae species that are associated with mango fruit rot. Correct identification of these pathogens is helpful for more effective management of fruit rot disease of mango. Further screening of effective fungicides and understanding of the epidemiology of these fungal pathogens will help to reduce financial loss to the mango industry in Taiwan.

Acknowledgement. This study was supported by the Council of Agriculture of Taiwan. We thank S. L. Hsu and S. Y. Lai for their excellent technical supports.

LITERATURE CITED

Abdollahzadeh, J., A. Javadi, E. Mohammadi Goltapeh, R. Zare, and A.J.L. Phillips. 2010. Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. Persoonia **25:** 1-10.

Alves, A., A.J.L. Phillips, I. Henriques, and A. Correia. 2005. Evaluation of amplified ribosomal DNA restriction analysis as a method for the identification of *Botryosphaeria* species. FEMS Microbiol. Lett. **245:** 221-229.

Alves, A, P.W. Crous, A. Correia, and A.J.L. Phillips. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Div. **28:** 1-13.

Amponsah, N.T., E.E. Jones, H.J. Ridgway, and M.V. Jaspers. 2011. Identification, potential inoculum sources and pathogenicity of Botryosphaeriaceous species associated with grapevine dieback disease in New Zealand. Eur. J. Plant Pathol. **131:** 467-482.

Arauz, L.F. 2000. Mango anthracnose: economic impact and current options for integrated management. Plant Dis. **84:** 600-611.

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

Crous, P.W. and J.Z. Groenewald. 2005. Hosts, species and genotypes: opinions versus data. Austral. Plant Pathol. **34:** 463-470.

Crous, P.W., B. Slippers, M.J. Wingfield, J. Rheeder, W.F.O. Marasas, A.J.L. Philips, A. Alves, T. Burgess, P. Barber, and J. Z. Groenewald. 2006. Phylogenetic lineages in the Botryosphaeriaceae. Stud. Mycol. **55:** 235-253.

de Oliveira Costa, V.S., S.J. Michereff, R.B. Martins, C.A.T. Gava, E.S.G. Mizubuti, and M.P.S. Câmara. 2010. Species of Botryosphaeriaceae associated on mango in Brazil. Eur. J. Plant Pathol. **127:** 509-519.

de Macedo, D.M. and R.W. Barreto. 2008. First record of *Botryosphaeria ribis* associated with leaf spots on *Magonlia* aff. *Candollei* in Brazil. Brazil. J. Microbiol. **39:** 321-324.

Deman, S., P.W. Crous, J.E. Taylor, J.C. Kang, I. Pascoe, and M.J. Wingfield. 2000. An overview of the taxonomic history of *Botryosphaeria*, and re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Stud. Mycol. **45:** 129-140.

Damm, U., P.W. Crous, and P.H. Fourie. 2007. Botryosphaeriaceae as potential pathogens of Prunus in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia **99:** 664-680.

De Wet, J., B. Slippers, O. Preisig, B.D. Wingfield, and M.J. Wingfield. 2008. Phylogeny of the Botryosphaeriaceae reveals pattern of host association. Mol. Phylogen. Evol. **46:** 116-126.

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

Hsu, S.T., T.T. Chang, C.A. Chang, J.L. Tsai, and T.T. Tsai (eds.). 2002. List of plant diseases in Taiwan, 4th ed. The Phytopathological Society of the Republic of China, Taiwan, 386 pp.

Jacobs, K.A. and S.A. Rehner. 1998. Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. Mycologia **90:** 601-610.

Javier-Alva, J., D. Gramaje, L.A. Alvarez, and J. Armengol. 2009. First report of *Neofusicoccum parvum* associated with dieback of mango trees in Peru. Plant Dis. **93:** 426.

Johnson, G.I. 2008. Status of mango postharvest disease management R&D: Options and solutions for the Australian mango industry. Horticulture Australia Final report for project MG08017, pp. 1-130.

Johnson, G.I. and A.W. Cooke. 1991. Stem end rot mango in Australia: cause and control. ISHS Acta. Hort. **291:** 288-295.

Ko, Y., C.W. Liu, C.Y. Chen, S. Maruthasalam, and C.H. Lin. 2009. First report of stem-end rot of mango caused by *Phomopsis mangiferae* in Taiwan. Plant Dis. **93:** 764.

Lazzizera, C., S. Frisullo, A. Alves, and A.J.L. Phillips. 2008. Morphology, phylogeny and pathogenicity of *Botryosphaeria* and *Neofusicoccum* species associated with drupe rot of olives in southern Italy. Plant Pathol. **57:** 948-956.

Liao, J.X. 1975. Mango disease in Taiwan-stem-end rot. Scientific Agriculture **23:** 415-416.

Marincowitz, S., J.Z. Groenewald, M.J. Wingfield, and P.W. Crous. 2008. Species of Botryosphaeriaceae occurring on Proteaceae. Persoonia **21:** 111-118.

Ni, H.F., R.F. Liou, T.H. Hung, R.S. Chen, and H.R. Yang. 2009. First report of a fruit rot disease of avocado caused by *Neofusicoccum mangiferae*. Plant Dis. **93:** 760.

Niekerk, J.M., W. van Bester, F. Halleen, P.W. Crous, and P.H.

Fourie. 2011. The distribution and symptomatology of grapevine trunk disease pathogens are influenced by climate. Phytopath. Medit. **50 (sup.):** 98-111.

Phillips, A.J.L. 2002. *Botryosphaeria* species associated with diseases of grapevines in Portugal. Phytopathol. Mediterr. **41:** 3-18.

Phillips, A. J. L., I. C. Rumbos, A. Alves, and A. Correia. 2005. Morphology and phylogeny of *Botryosphaeria dothidea* causing fruit rot of olives. Mycopathologia **159:** 433-439.

Ploetz, R.C., G.A. Zentmyer, W.T. Nishijima, K.G. Rohrbach, and H.D. Ohr. 1994. Compendium of Tropical Fruit Diseases. American Phytopathological Society Press. St. Paul, Minnesota, 118 pp.

Punithalingam, E. 1980. Plant diseases attributed to *Botryodiplodia theobromae*. *In* Biblioteca Mycologica. J. Cramer, Berlin.

Sakalidis, M.L., J.D. Ray, V. Lanoiselet, G.E.S. Hardy, and T.I. Burgess. 2011. Pathogenic Botryosphaeriaceae associated with *Mangifera indica* in the Kimberley region of western Australia. Eur. J. Plant Pathol. **130:** 379-391.

Shen, Y.M., C.H. Chao, and H.L. Liu. 2010. First report of *Neofusicoccum parvum* associated with stem canker and dieback of Asian pear trees in Taiwan. Plant Dis. **94:** 1062.

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

Slippers, B., G.I. Johnson, P.W. Crous, T.A. Coutinho, B.D. Wingfield, and M.J. Wingfield. 2005. Phylogenetic and morphological re-evaluation of the *Botryosphaeria* species causing diseases of *Mangifera indica* in Australia. Mycologia **97:** 99-110.

Slippers, B., P.W. Crous, S. Denman, T.A. Coutinho, B.D. Wingfield, and M.J. Wingfield. 2004. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia **96:** 83-101.

Smith, D.R. and G.R. Stanosz. 2001. Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. Mycologia **93:** 505-515.

Smith, H., M.J. Wingfield, P.W. Crous, and T.A. Coutinho. 1996. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. S. Afr. J. Bot. **62:** 86-88.

Taylor, A., G.E. St J. Hardy, P. Wood, and T. Burgess. 2005. Identification and pathogenicity of *Botryosphaeria* species associated with grapevine decline in Western Australia. Austral. Plant Pathol. **34:** 187-195.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. **25:** 4876-4882.

Úrbez-Torres, J.R., G.M. Leavitt, J.C. Guerrero, J. Guevara, and W.D. Gubler. 2008. Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the causal agents of bot canker disease of grapevines in Mexico. Plant Dis. **92:** 519-529.

Úrbez-Torres, J.R., G.M. Leavitt, T.M. Voegel, and W.D. Gubler. 2006. Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. Plant Dis. **90:** 1490-1503.

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

Wang, C.L. and H.Y. Hsieh. 2006. Occurrence and Pathogenicity of Stem Canker of Guava in Taiwan Caused by *Botryosphaeria rhodina*. Plant Pathol. Bull. **15:** 219-230.

Wang, F., L. Zhao, and G. Li. 2011. Identification and characterization of *Botryosphaeria* spp. causing gummosis of peach trees in Hubei Province, central China. Plant Dis. **95:** 1378-1384.

Wang, H., M. Qi, and A.J. Culter. 1993. A simple method of preparing plant samples for PCR. Nucl. Acids Res. **21:** 4153-4154.

Wang, H.L., P.H. Chen, H.F. Ni, and R.S. Chen. 2007. Physiological characterization and screen of control chemicals for *Lasiodiplodia theobromae* of papaya. Plant Pathol. Bull. **16:** 71-77.

Wang, P.H., Y.S. Chen, M.J. Lin, Y.J. Tsou, and W.H. Ko. 2010. Severe decline of wax apple trees caused by *Fusarium solani* in northern Taiwan. Bot. Stud. **51:** 75-80.

White, T.J., T. Bruns, S. Lee, and J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. *In* M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White (eds.), PCR Protocols: A Guide to Methods and Applications. Academics Press. San Diego, pp. 315-322.

Yang, H.C. and L.S. Leu. 1988. The morphological and physiological characteristics of the causal agent of mango anthracnose, *Colletotrichum gloeosporioides* Penzig. Plant Prot. Bull. **30:** 323-336.

Yu, L., X.L. Chen, L.L. Gao, H.R. Chen, and Q. Huang. 2009. First report of *Botryosphaeria dothidea* causing canker and shoot blight of Eucalyptus in China. Plant Dis. **93:** 764.

Botryosphaeriaceae 在臺灣引起之檬果果腐病：病原種類鑑定及其病原性

倪蕙芳[1,2] 楊宏仁[1] 陳瑞祥[3] 劉瑞芬[2] 洪挺軒[2]

[1] 農業試驗所嘉義農業試驗分所 植物保護系

[2] 國立臺灣大學 植物病理與微生物學系

[3] 國立嘉義大學 生化科技學系

檬果為臺灣重要果樹之一，近年來於採收後果實上發生嚴重之果腐病。為了瞭解其發生情形及病原菌種類，本研究於 2009-2011 年在臺灣三個主要檬果產區 (枋山、玉井及官田) 進行果腐病害調查及病原分離，結果顯示其病害發生率自 18.7% 至 58.1% 不等，其中官田地區之發生率更高於玉井及枋山地區。自果腐病斑所分離之病原菌經以形態特性及 ITS, β-tubulin (TUB) 與 EF1-α 等基因之序列鑑定後，共發現四種葡萄座腔菌科病原菌，包括 *Lasiodiplodia theobromae*, *Neofusicoccum mangiferae*, *N. parvum* 及 *Fusicoccum aesculi*。不論是直接接種或先製造傷口再接種，這四種病原菌都可成功感染檬果果實，顯示其均具有病原性，而且 *L. theobromae* 之致病力較其它三種病原菌還強。當以生長於檬果樹之未成熟果實進行接種試驗時，發現不需製造傷口，病原菌之分生胞子即可直接感染果實，而且採收之果實在成熟後也會出現果腐病斑。本研究證明檬果果腐病乃由 *L. theobromae*, *F. aesculi*, *N. mangiferae* 及 *N. parvum* 引起，且其分生胞子可能為田間之主要感染源。

關鍵詞：檬果；葡萄座腔菌科；果腐病。

Table S1. Sequences of Botryosphaeriaceae species from GenBank used in the phylogenetic analysis.

Species	GenBank Accession No.		
	ITS	EF1-α	TUB
Lasiodiplodia theobromae	AY640255	AY236901	AY236930
	DQ458891	AY640258	EU673110
	GQ502452	GQ980005	GU056847
	GQ502455		GU056853
Neofusicoccum parvum	AY259098	AY573221	AY615169
	AY615182	DQ487158	EU673095
	GQ861435	GQ985315	GU062770
N. mangiferae	AY615186	DQ093221	AY615172
	DQ316081		AY615173
Fusicoccum aesculi	AY615191	EF585562	AY615178
(*Botryosphaeria dothidea*)	EF638769	EF638727	EU673106

Table S2. Dimensions of conidia of selected isolates of *Botryosphaeriaceae* species collected from mango fruits with fruit rot.

Isolate	Identity	Conidium length (μm ± SD)	Conidium width (μm ± SD)	L/W
B961	*Lasiodiplodia theobromae*	24.35±1.48(50)[a]	12.47±1.40	1.98±0.32
B965	*L. theobromae*	23.40±1.62(50)	12.75±0.95	1.84±0.18
B838	*L. theobromae*	24.86±1.38(50)	14.42±0.78	1.73±0.12
B852	*L. theobromae*	24.99±1.90(50)	15.59±0.87	1.61±0.15
B918	*L. theobromae*	23.49±1.22(50)	12.90±0.66	1.82±0.13
B902	*L. theobromae*	24.73±1.98(50)	13.35±0.99	1.86±0.18
B878	*L. theobromae*	24.77±1.19(50)	13.07±0.77	1.87±0.16
B607	*L. theobromae*	27.18±1.86(50)	15.08±1.75	1.82±0.21
B845	*Neofusicoccum parvum*	15.85±1.16(58)	4.49±0.11	3.52±0.30
B946	*N. parvum*	16.54±1.63(50)	5.57±0.40	2.97±0.28
B1314	*N. parvum*	17.71±1.68(56)	6.61±0.80	2.70±2.24
B794	*N. parvum*	18.87±2.11(50)	5.25±0.65	3.65±0.65
B1260	*N. parvum*	19.25±1.53(50)	5.97±0.54	3.26±0.37
B1272	*N. parvum*	19.24±2.23(50)	6.13±1.52	3.26±0.40
B1296	*N. parvum*	18.50±1.65(52)	5.07±0.57	3.68±0.52
B1307	*N. parvum*	19.08±1.34(50)	5.68±0.48	3.39±0.47
B809	*N. mangiferae*	12.01±0.94(50)	6.35±0.55	1.86±0.20
B793	*N. mangiferae*	11.98±0.80(50)	6.25±0.47	1.92±0.15
B808	*N. mangiferae*	12.26±0.77(60)	6.37±0.34	1.93±0.13
B979	*N. mangiferae*	12.50±1.00(50)	6.42±0.48	1.95±0.15
B763	*N. mangiferae*	12.93±0.93(50)	6.98±0.40	1.85±0.13
B964	*Fusicoccum aesculi*	21.56±2.18(50)	6.40±2.22	3.50±0.53
B811	*F. aesculi*	19.31±1.59(50)	6.10±0.36	3.17±0.26
B844	*F. aesculi*	19.86±1.20(50)	6.38±0.36	3.12±0.24
B922	*F. aesculi*	22.10±1.92(50)	6.28±0.30	3.52±0.30
B833	*F. aesculi*	18.72±2.70(50)	6.14±0.36	3.05±0.43
B801	*F. aesculi*	19.67±1.04(50)	5.72±0.38	3.46±0.31
B932	*F. aesculi*	21.34±1.47(50)	6.63±0.70	3.26±0.41
B1113	*F. aesculi*	20.51±1.31(54)	5.92±0.32	3.48±0.31

[a] Numerals inside the parenthesis indicate the number of conidia used for the measurement of length and width.