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Research Article

Anthracnose of Pitaya (*Hylocereus* spp.) Caused by *Colletotrichum* spp., A New Postharvest Disease in Taiwan

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Abstract

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Pitaya (*Hylocereus undatus*, *H. polyrhizus*, and *H. costaricensis*) has been widely planted as a fruit crop in Taiwan in the past three decades. Field survey in pitaya orchards during 2009 to 2013 showed that anthracnose of pitaya caused by *Colletotrichum* spp. was one of the major postharvest diseases in Taiwan. Disease symptoms encompassed brown colored sunken spots with or without water-soaked necrosis on the harvested fruits skin after storing at room temperature for 5–10 d, which apparently shortening the marketable period and reducing the price of the harvested fruits. Based on morphological characteristics and ITS region sequences, at least three species of *Colletotrichum* isolated from diseased fruits of pitaya collected from orchards in Taiwan were identified, including *C. truncatum* and 2 species within each respective *C. gloeosporioides* and *C. boninense* species complex, with isolation frequencies of 21.8, 73.4, and 4.8%, respectively, during 2009 to 2013. However, to precisely discriminate the *Collectotrichum* species within each respective *C. gloeosporioides* and *C. boninense* species complex, further multi-locus phylogenetic analyses are mandatory in the future. This is the first report of anthracnose caused by *Colletotrichum* spp. as an important postharvest disease of pitaya in Taiwan.

Key words: *Hylocereus* spp., Postharvest disease, Anthracnose, *Colletotrichum* spp.

INTRODUCTION

Pitaya [*Hylocereus undatus* (Haworth) Britton & Rose, *H. polyrhizus* (Weber) Britt. & Rose, and *H. costaricensis* (F.A.C. Weber) Britton & Rose], also known as dragon fruit, has become an economically important fruit crop, and has been widely cultivated in Taiwan in the past three decades (Liu & Liu 2015). The period for fruit production of pitaya was mainly in the summer months (Jun. to Sep.), with average temperature 28–32℃. According to our observation, severe economic losses could occurr during storage, transportation, and marketing of harvested pitaya fruits which were under room temperature (RT, 24–32℃) and high humidity, conditions conducive to the development of postharvest diseases.

A postharvest disease with light brown lesions on pitaya fruits caused by *Bipolaris cactivora* (Petr.) Alcorn was previously reported in Taiwan by Wang & Lin (2005). However,

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symptoms on some specimens of pitaya fruits collected in 2009 in the pitaya production orchards in Taiwan were different from the previously reported postharvest disease of pitaya caused by *B. cactivora* (Wang & Lin 2005). Further disease survey in this paper revealed that anthracnose was frequently found on harvested fruits of pitaya stored at room temperature in Taiwan.

The objective of this study was to report anthracnose as a new postharvest disease of pitaya in Taiwan, based on disease survey, isolation and identification of the causal organisms, conducting pathogenicity tests to fulfill Kock's postulates, examining of morphological traits and rDNA ITS sequences blasting and preliminary phylogenetic analysis.

MATERIALS AND METHODS

Field survey on postharvest diseases of pitaya

Field surveys on postharvest diseases of pitaya, *H. undatus*, *H. polyrhizus*, and *H. costaricensis*, were conducted from 2009 to 2013. During the experimental period, there were 12 pitaya orchards in total in the major pitaya production areas in Taiwan, including two orchards in each county of Changhua, Hualien, Nantou, and Pingtung, and one orchard in each county of Chiayi, Taichung, Tainan, and Yilan. Thirty freshly harvested fruits of pitaya without visible lesions were collected from each orchard and stored at room temperature (RT) for 5 to 15 days during fruit production period (Jun. to Sep.). Fruits showing symptoms of brown colored sunken spots were recorded, followed by isolation and identification of the disease causal agents.

Isolation and maintenance of the disease causal agents

Tissues (0.5 cm × 0.5 cm × 0.2 cm; L × W × H) were excised from lesions of diseased pitaya fruits, and immersed in 0.5% (v/v) NaOCl for 30 s, then overlaid on potato dextrose agar (PDA; Difco Co., USA) in Petri dishes, 1 diseased tissue/dish. The plates were incubated for 3 d at 22–24℃ under 12 h/12 h light/dark period. Once the hyphae emerged from tissues and spread onto agar media, small agar blocks containing hyphal tips were cut and transferred onto PDA media. By colony features on agar media and conidial morphology under microscopy, the fungi isolates preliminarily identified as *Colletotrichum* spp were further purified by single-spore isolation technique to establish axenic culture. A total of 124 single spore isolates of *Colletotrichum* spp. were obtained and cultured on PDA at 22–24℃ under 12 h/12 h light/dark period for 7 d. Agar plugs (0.5 cm × 0.5 cm × 0.2 cm; L × W × H) containing mycelial mats were excised from the edge of 5-day-old colony on PDA in Petri dishes, and immersed in mineral oil (Sigma-Aldrich., USA) in plastic tubes (7–10 plugs/tube), sealed with parafilm™, and kept at 22–24℃ for long-term storage. For each working culture preparation, one mycelial plug of each isolate was retrieved from one long-term storage vial, subcultured on PDA plate, and incubated at 22–24℃ under 12 h/12 h light/dark period for 7 d.

Morphology and growth of *Colletotrichum* spp.

To study the effects of temperature on growth of *Colletotrichum* isolates F211117, F211178, and F210016, agar blocks (0.5 cm × 0.5 cm × 0.2 cm; L × W × H) containing mycelial mats were cut from the colony edge of 5-day-old PDA cultures in Petri dishes and inoculated on fresh PDA Petri dishes center, 1 block/dish. The dishes were sealed with parafilm™ and incubated differently at 4 to 40℃ with 4℃ intervals in dark. Colony diameter of each dish was measured daily for a period of 7 d. Growth rate (mm/day) of each colony was calculated by: (colony diameter of the 7th day – colony diameter of the 1st day)/6 days. Experiments were repeated twice, each isolate with three replicates at each temperature.

For conidial propagation, the 3 *Colletotrichum* isolates were grown on PDA in Petri

dishes at 22–24℃ under 12 h/12 h light/dark period. After incubation for 7 d, conidia were harvested by washing the colony with sterilized double distilled water (ddH_2O), 15 mL/dish. To study conidia and appressoria morphological characteristics of the Collectotrichum species, the conidia were suspended in 0.5% V8 juice (Campbell Co., USA), pipetted onto glass slide, 20 μL/slide, then superposed on wet tissue in a Petri dish sealed with parafilm™, and incubated at 24℃ in dark for 20 h or 60 h. Totally, 100 conidia and 100 appressoria were randomly selected and examined for color, size, shape and length/width (L/W) ratio under a compound light microscope (Olympus BX40).

rDNA ITS blasting and phylogentic analysis

Mycelial mats of *Colletotrichum* isolates F211117, F211178, and F210016, were collected from 5-day-old PDA cultures and used for extraction of DNA, using Genomic DNA Purification Kit (GeneMark Technology Co., Taiwan) according to the manufacturer's protocol. The primers ITS5/ITS4 for internal transcribed spacer (ITS; White *et al*. 1990) were used for polymerase chain reaction (PCR) under the recommended PCR conditions. The PCR amplicons were sequenced in both directions by the Seeing Bioscience Co., Taipei, Taiwan. DNA sequences were assembled by Vector NTI software v. 10.0 (InforMax Inc., USA). The sequences were uploaded to GenBank and NCBI BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to blast the best hit. Multiple sequence alignment based on ITS sequences was performed initially by Clustal Omega. A distance tree generated by the neighbour-joining (NJ) algorithm estimated by bootstrapping with 1,000 replicates was built by MEGA v.7.0 (Kumar *et al*. 2016).

Pathogenicity test

To prove the pathogenicity of the 3 *Colletotrichum* isolates, F211117, F211178, and F210016, Koch's postulates tests were conducted using freshly harvested fruits of *H. undatus*. In brief, twenty days prior to harvesting, the fruits selected for pathogenicity tests were covered individually with paper bags mainly to prevent latent infection by *Collectotrichum* pathogens during fruit growing stage in the orchard. Conidia were collected from 7-day-old PDA cultures in Petri dishes by washing the colonies with sterilized ddH_2O. The conidial suspension was filtered through 8-layer sterilized gauze, and adjusted concentration to 1×10^6 conidia/mL using sterilized ddH_2O. For each isolate, cotton mats, about 1 cm in diameter, were soaked in conidial suspension as inoculums. Two pitaya fruits of each set were respectively inoculated two sites with or without wounding. Twenty wounded spots were created by puncturing the fruit skin about 1 mm in depth with a bundle of 10-insect dissecting needles. Each site was covered with one cotton mat mentioned above which absorbed the conidial suspension. Instead, cotton mats soaked with sterilized ddH_2O was served as control. The inoculated fruits were placed in a plastic box moisture chamber (32 cm × 20.5 cm × 10 cm, L × W × H) bottom overlaid with moist paper towel, 2 fruits in a chamber. The chamber were covered with lid for maintaining high relative humidity (> 95%), and incubated at RT for 4 to 6 d, thereafter examined the presence of lesions on each fruit. Lesion showing anthracnose symptoms on each inoculation site were excised for pathogen reisolation by the methods described previously. Disease incidence (DI) was calculated by the formula: DI (%) = (No. of infected sites)/(Total No. of inoculated sites) × 100 (%). The inoculation tests were repeated two times.

RESULTS

Disease survey and symptoms on pitaya fruits in storage

The survey results from 2009 to 2013 showed that anthracnose disease was the most important postharvest disease of pitaya in

Taiwan. Survey the total 360 fruits, collected from 12 different orchards and stored at RT, 73.1% of the fruit samples showed symptoms of anthracnose. Symptoms first appeared on cutting sites or scales of pitaya fruits stored at RT for 5 to 10 days. Light brown colored, water-soaked sunken spots were formed on the surface of diseased fruits, producing of grayish to pinkish spore masses on some of the lesions under humid conditions (Fig. 1). Lesions on fruits often expanded and coalesced, causing fruit scales withering and fruits watery and softening. Beside the typical symptoms mentioned above (Fig. 1A), some diseased fruits produced black microsclerotia, setae and acervuli (Fig. 1B), and some showed light to dark brown lesions (Fig. 1C).

Isolation and preliminary identification of the fungi isolates

In addition to the 12 orchards investigated during the field survey in 2009 to 2013, pitaya fruits from other orchards and market showed anthracnose symptoms were also selected for isolation of causal agents. A total of 124 fungal isolates were isolated from pitaya fruits and established pure cultures by single-spore isolation technique. All the 124 isolates were identified as *Colletotrichum* spp. according to the colony features on PDA media and conidial morphology. Three major types of conidia were found among these *Colletotrichum* isolates (Table 1). Isolates belonging to conidial type 1 produced straight cylindrical shaped conidia, average L/W ratio > 2.5 and < 3.5; isolates belonging to conidial type 2 produced long falcate conidia, average L/W ratio > 6.5 and < 7.5; isolates belonging to conidial type 3 produced straight cylindrical shaped conidia, average L/W ratio > 1.5 and < 2.5, which were shorter and wider than those of conidial type 1 in general. Of the 124 single-spore isolates of *Colletotrichum* from pitaya fruits, the isolation frequencies of conidial types 1, 2, and 3 were 73.4, 21.8 and 4.8%, respectively (Table 1).

Colonial and microscopic characteristics of *Colletotrichum* isolates

Three *Colletotrichum* isolates, F211117, F211178, and F210016, belonging to conidial types 1, 2 and 3, respectively, were selected for subsequent studies. The three isolates grown on PDA at RT for 7 days showed differences in colonial morphology. Colonies of isolate F211117 were greyish white to dark grey, formed aerial mycelia initially (Fig. 2A), later produced massive orange color conidia. Colonies of isolate F211178 were greyish to greenish white, cottony to floccose (Fig. 2B). Colonies of isolate F210016 were smooth and

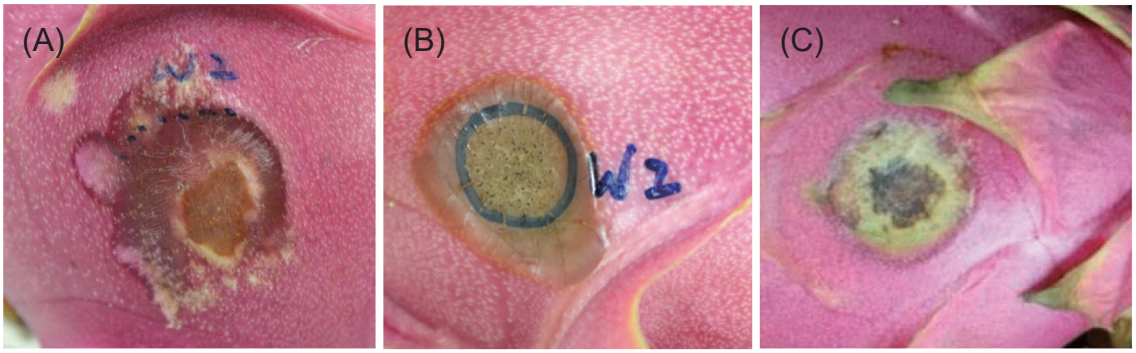

Fig. 1. Symptoms of anthracnose on pitaya fruits (*Hylocereus undatus*). Beside typical symptoms of brown, sunken, and severe water-soaking caused by *Colletotrichum gloeosporioides* isolate F211117 (A), *C. truncatum* isolate F211178 caused symptoms of black spot and formed microsclerotia in the inoculation site (B), and *C. boninense* F210016 caused symptoms of light to dark brown lesions with mild water-soaking (C). Pitaya fruits were inoculated, using conidial suspension (1×10^6 conidia mL^{-1}) of respective isolates, and incubated at RT for 4 to 6 d.

Table 1. Conidial types and morphological characteristics of *Colletotrichum* isolates from diseased fruits of pitaya in Taiwan, 2009–2013[z].

Conidial type	No. of isolate	Ratio (%)	Character of conidia[y]
1	91	73.4	Straight and cylindrical, > 2.5 and < 3.5 in avg. L/W ratio
2	27	21.8	Falcate, > 6.5 and < 7.5 in avg. L/W ratio
3	6	4.8	Straight and cylindrical, < 1.5 and < 2.5 in avg. L/W ratio
Total	124	100.0	

[z] Pitaya fruits were collected from orchards in the major pitaya-producing counties in Taiwan. Fruits developed anthracnose symptoms during the period of storage at room temperature (RT) for 1–15 d were used for isolation of *Colletotrichum*.
[y] Conidia were harvested from 7-day-old PDA cultures under 12 h/12 h photoperiod at 22–24℃.

flat, white to salmon (Fig. 2C)

Morphological features of conidia produced on 7-day-old PDA cultures and appressoria produced in 0.5% V8 juice were compared. In isolate F211117, conidia were hyaline, aseptate, straight or cylindrical, round apex, truncate base, (14.9) 12.5–17.8 × (5.2)–3.6–7 µm [(avg.) min.–max., length × width), and (2.9) 2.1–4.5 (L/W) (Fig. 3A). Appressoria were sepia to light brown, oval or globular, (7.8) 4.8–9.8 × (6.3) 5.1–8.4 µm (Fig. 3B). In isolate F211178, conidia were longer than those of conidial type 1 and type 3, (24.7) 19.5–30 × (3.8) 2.5–5 µm and (6.5) 4.5–11.5 (L/W), and the conidial shape were crescent with abrupt apex and truncate base (Fig. 3C). Appressoria were smooth, oval, at first 20 h, (7.7) 5.7–10.2 × (5.4) 4.7–6.7 µm (Fig. 3D), at 60 h, some of the appressoria turned into clavate to irregular, in groups or dense clusters (Fig. 3G). In isolate F210016, conidia were straight, cylindrical with round apex and base (Fig. 3E), (14.2) 12.5–17.5 × (6.1) 5.0–7.5 µm, and (2.4) 1.6–3.5 (L/W). Appressoria were oval or irregular, and (10.2) 6.0–22.3 × (8.1) 4.7–16.7 µm (Fig. 3F). Furthermore, isolate F211178 produced setae on PDA cultures as well as on lesions of diseased fruits with abrupt tip (Fig. 3H). However, setae were absent in the isolates F211117 and F210016.

Temperatures that affect the hyphal growth rate varied with these three anthracnose pathogens (Fig. 4). The range of minimum and maximum temperatures for hyphal growth were 4–8 and 36–40℃, respectively, with optimum

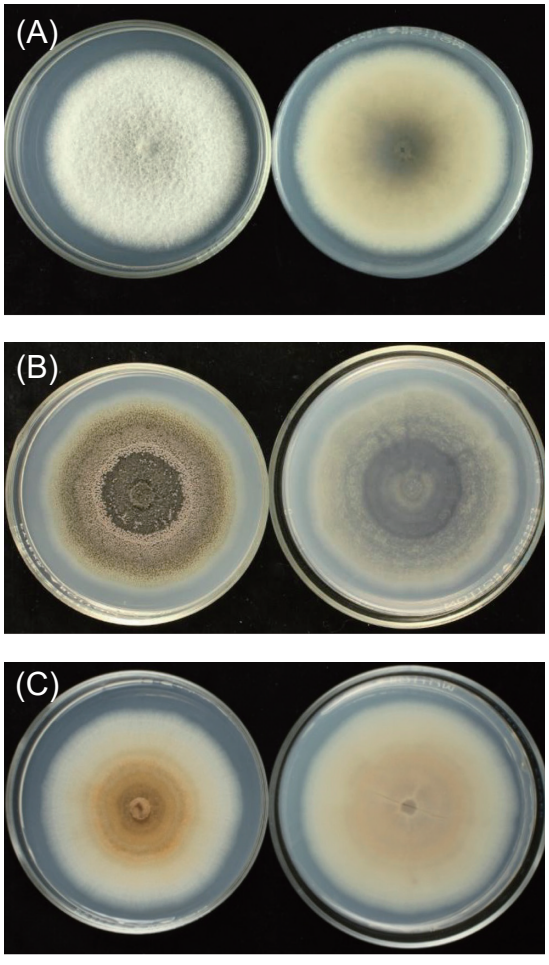

Fig. 2. Top and reverse colony features of *Colletotrichum gloeosporioides* isolate F211117 (A), *C. truncatum* isolate F211178 (B), and *C. boninense* isolate F210016 (C). The cultures were grown on PDA at 22–24℃ under 12 h/12 h light/dark period for 7 to 9 d. Distinct differences in colony color and texture among the three *Colletotrichum* species were noted. (Left row = top view; Right row = bottom view)

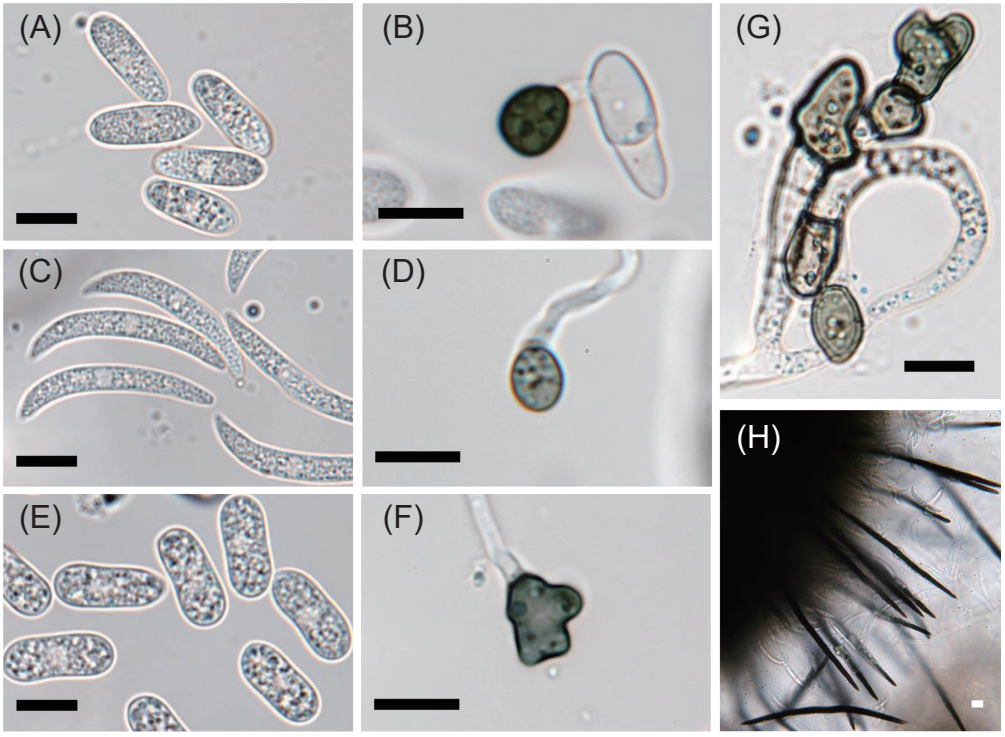

Fig. 3. Conidia (A, C, E), appressoria (B, D, F, G), and setae (H) of *Colletotrichum gloeosporioides* isolate F211117 (A, B), *C. truncatum* isolate F211178 (C, D, G, H), and *C. boninense* isolate F210016 (E, F). The cultures were grown on PDA for 7 d for formation of conidia, and subsequently submerged in 0.5% V8 juice for 20 h (D, E, F) for formation of appressoria. Some of the apporessoria of *C. truncatum* isolate F211178 turned into different shapes at 60 h (G). Differences in morphology of conidia and shape of appressoria among the three *Colletotrichum* species were noted. Setae of *C. truncatum* isolate F211178 (H) present, whereas were absent for the isolates F211117 and F210016, cultured on PDA at 22–24℃ under 12 h/12 h light/dark period for 14 d. (Bar = 10 μm)

temperature of 28–32℃ (Fig. 4).

Based on the morphological characteristics, including size and shape of conidia and appressoria (Figs. 2 and 3, Table 1) and capacity of setae formation (Fig. 2G), also compared with the description and circumscription of previous literatures, the *Colletotrichum* isolates F211117, F211178 and F210016 were identified as *C. gloeosporioides* (Sutton 1992; Weir *et al*. 2012), *C. truncatum* (Damm *et al*. 2009; Guo *et al*. 2014), and *C. boninense* (Moriwaki *et al*. 2003; Damm *et al*. 2012), respectively.

Blasting rDNA ITS sequences of the *Colletotrichum* isolates

The rDNA ITS regions of the three *Colletotrichum* isolates, F211117 (conidial type 1), F211178 (conidial type 2) and F210016 (conidial type 3), were sequenced and deposited in NCBI GenBank, with accession numbers: KM881568–KX197406 (Table 1). Blast the GenBank database with the uploading ITS region sequences of isolates F211117, F211178 and F210016, query revealed the best hits of 99.8, 100 and 100% identity to those annotated ITS regions of *C. gloeosporioides* (Penz.) Penz. & Sacc., *C. truncatum* (Schwein.) Andrus & W.D. Moore, and *C. boninense* Moriwaki, Toy. Sato & Tsukib, respectively (Table 2).

Additionally, ITS regions of other *Colletotrichum* isolates were also sequenced (Table 2). The other 11 isolates belonging to conidial type 1 showed 99.0–99.8% identity to isolates F211117 (conidial type 1). Again, these

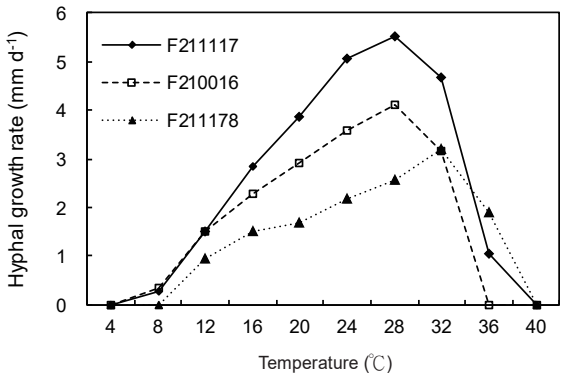

Fig. 4. Effect of temperatures on colony growth rate of *Colletotrichum gloeosporioides* isolate F211117, *C. truncatum* isolate F211178, and *C. boninense* isolate F210016. Colony growth rate was measured daily on PDA cultures for 7 d.

Table 2. Comparison of ITS region sequences and blast results of *Colletotrichum* isolates belonging to different conidial types.

Isolate	GenBank accession no.	Identity to ref. seq., respectively (%)	Geographic origin	Best hit accession no.[z], identity (%)
Conidial type 1, *C. gloeosporioides*				
F211117	KM881568, 598 bp	As ref. seq. of type 1	Taichung	AY266389.1, 99.8
F211156	KX197384, 599 bp	99.0	Hualien	EU552111.1, 100
F210004	KX197385, 599 bp	99.2	Nantou	AY266373.1, 100
F210042	KX197386, 599 bp	99.3	Nantou	KT966505.1, 99.8
F210052	KX197387, 599 bp	99.3	Nantou	KT390195.1, 100
F210008	KX197388, 598 bp	99.8	Nantou	AY266389.1, 100
F210035	KX197389, 598 bp	99.8	Nantou	AY266389.1, 100
F210332	KX197390, 598 bp	99.8	Hualien	AY266389.1, 100
F210203	KX197391, 598 bp	99.7	Taichung	AY266389.1, 99.8
F210024	KX197392, 598 bp	99.5	Taichung	AY266378.1, 100
F210199	KX197393, 598 bp	99.5	Taichung	AY266378.1, 100
F210235	KX197394, 598 bp	99.5	Taichung	AY266378.1, 100
Conidial type 2, *C. truncatum*				
F211178	KM881567, 604 bp	As ref. seq. of type 2	Pingtung	AY266370.1, 100
F211109	KX197395, 604 bp	99.8	Taichung	AY266372.1, 100
F211113	KX197396, 604 bp	99.8	Taichung	AY266372.1, 100
F212206	KX197397, 604 bp	99.8	Taichung	AY266372.1, 100
F210115	KX197398, 604 bp	100.0	Taichung	AY266370.1, 100
F211165	KX197399, 604 bp	100.0	Nantou	AY266370.1, 100
F212051	KX197400, 604 bp	100.0	Nantou	AY266370.1, 100
F213095	KX197401, 604 bp	100.0	Nantou	AY266370.1, 100
F213119	KX197402, 604 bp	100.0	Changhua	AY266370.1, 100
F213132	KX197403, 604 bp	100.0	Nantou	AY266370.1, 100
F213202	KX197404, 604 bp	100.0	Nantou	AY266370.1, 100
Conidial type 3, *C. boninense*				
F210016	KM881566, 618 bp	As ref. seq. of type 3	Taichung	JX258728.1, 100
F212054	KX197405, 617 bp	99.7	Nantou	JX258728.1, 99.7
F212102	KX197406, 618 bp	100.0	Taichung	JX258728.1, 100

[z] The best hit accession numbers of GenBank listed in conidial types 1, 2, and 3 were annotated as ITS region sequences of *Colletotrichum gloeosporioides*, *C. trunctum*, and *C. boninense*, respectively.

sequences also showed best hit to those ITS regions annotated *C. gloeosporioides* individually. On the other hand, totally 10 isolates belonging to conidial type 2, and totally 2 isolates of conidial type 3, exhibited 99.8–100% and 99.7–100% identity to isolates F211178 (conidial type 2) and F210016 (conidial type 3), respectively. Coincidentally, the sequences of these isolates belonging to conidial types 2 and 3 were all hit the annotated *C. truncatum* and *C. boninense* in the GenBank, respectively. Preliminary phylogenetic analysis of the rDNA ITS region sequences of F211117 (conidial type 1), F211178 (conidial type 2) and F210016 (conidial type 3) and other ITS sequences of allied *Collectotrichum* species retrieved from GenBank database by maximum parsimony further confirmed the relatedness and identity of the *Colletotrichum* anthracnose pathogens isolated from dragon fruits in Taiwan (Fig. 5).

Pathogenicity of *Colletotrichum* isolates

Inoculation tests showed that all the three *Colletotrichum* isolates, F211117 (*C. gloeiosporoides*), F211178 (*C. truncatum*), and F210016 (*C. boninense*), from pitaya fruits were pathogenic to fruits of pitaya variety *H. undatus* (Table 3). Inoculated fruits showed up lesions after incubation at RT for 4 to 5 d. In

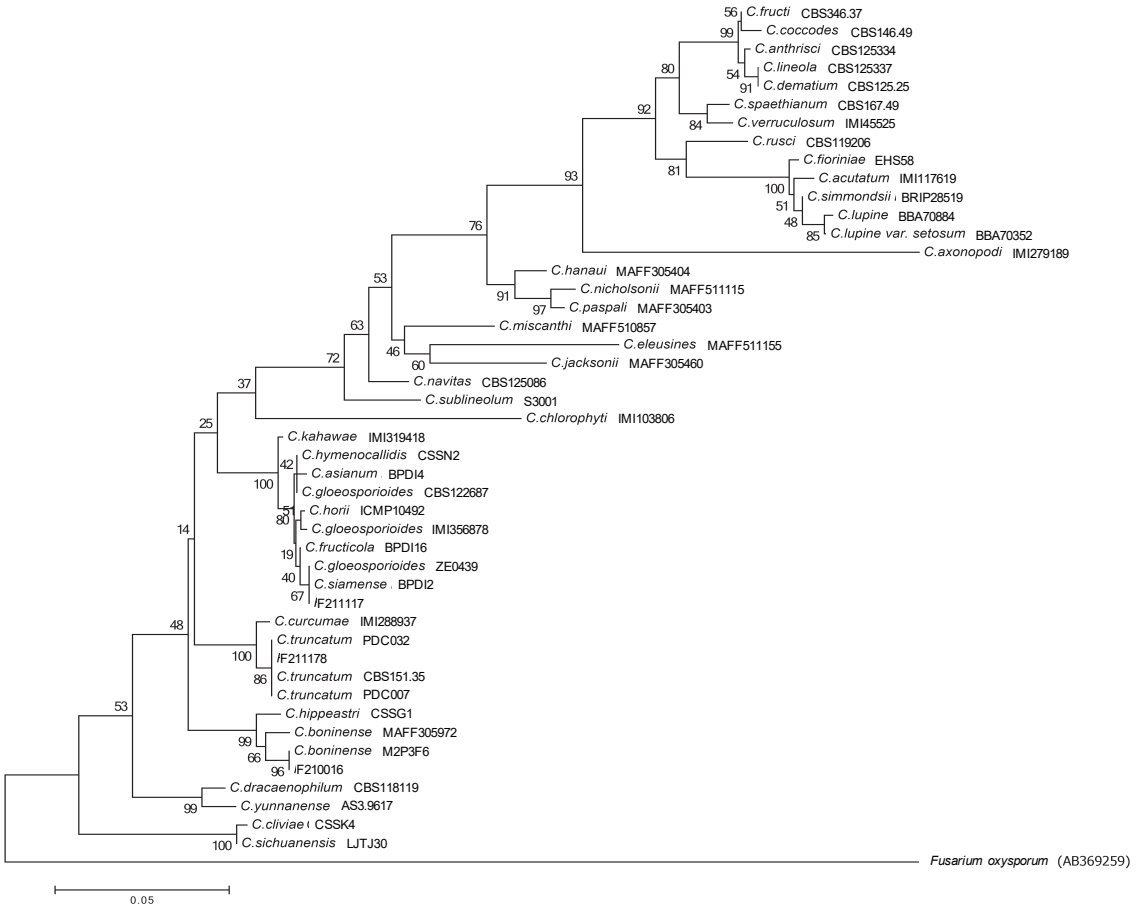

Fig. 5. A neighbour-joining tree based on the rDNA ITS region sequences, showing the phylogenetic relationships of F211117 (conidial type 1), F211178 (conidial type 2) and F210016 (conidial type 3) to other *Colletotrichum* species. The accession number of each sequence was indicated after allied *Collectotrichum* species and *Fusarium oxysporum*.

contrast to unwounding, wounding treatment substantially increased susceptibility of pitaya fruits to the three *Colletotrichum* isolates (Table 3). The range of disease incidences of unwounded pitaya fruits were 25.0, 75.0 and 12.5% for the isolates F211117, F211178, and F210016, respectively, whereas 100% on wounded fruits for all the three isolates. The symptoms on inoculated pitaya fruits were identical to the symptoms on naturally infected pitaya fruits collected from orchards and stored at RT. The pathogenicity of the three isolates to pitaya variety *H. polyrhizus* was similar to those on pitaya variety *H. undatus* (data not shown).

DISCUSSION

Survey of pitaya fruits during 2009 to 2013 reveals that anthracnose caused by *Colletotrichum* spp. is one of the most important postharvest diseases of pitaya in Taiwan. Morphological characteristics, molecular features and phylogentic analysis, and pathogenicity tests further confirmed the identity of the three causal *Colletotrichum* species: *C. gloeosporioides*, *C. truncatum*, and *C. boninense* as causal organisms. This is the first report of pitaya fruit rot disease caused by three *Colletotrichum* spp. via Kock postulates in Taiwan.

Blasting all of the rDNA ITS region sequences of conidial type 1 on GenBank database showed the best hit to *C. gloeosporioides*, with identity ranging from 99.0% to 99.8% (Table 2). The identity derived from ITS sequences concurred with the morphological characteristics of the *Collectotrichum* species with type 1 conidia (Figs. 1A and 1B). Hence, in a broad sense based on genetics and biology, the *Collectotrichum* isolates F211117 isolated from dragon fruit rot in Taiwan matches the circumscription of *C. gloeosporioides* (Sutton 1992; Masyahit *et al*. 2009; Weir *et al*. 2012). Actually this specie is heterogenous in nature in terms of variation in features of colony, conidia, setae, appresorium and host specificity, etc. Thus, Sutton (1992) further separated 6 formae speciales of physiological taxa and one variety with *Glomerella* teleomorph of *C. gloeosporioides* from *C. gloeosporioide* species complex; Weir *et al*. (2012) documented even more forma specials of it. Furthermore, to resolve the limitation using only ITS rDNA marker to distinguish *C. gloeosporioides* isolates collected worldwide in the past 50 years, by using multigenes' sequences: actin (ACT), calmoludin (CAL), chitin synthase-1 (CHS-1), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), internal transcribed spacers (ITS), glutamine synthase (GS), manganese superoxide dismutase-2 (SOD-2) and beta-tubulin (TUB2), conducted phylogenetic analysis with Bayesian Inferernce methods, Weir *et al*. (2012) accepted 22 species plus one subspecies within the *C. gloeosporioides complex*. In similar status, contrast to traditional *C. gloeosporioides*, *C. boninense* was erected by Moriwaki *et al*. (2003) based on the morphological and molecular characterization of *Collectotrichum* isolated from six plant species in Pacific Cost

Table 3. Pathogenicity of isolates of *Colletotrichum* spp. on fruits of *Hylocereus undatus*[z].

Species	Isolate	Disease incidence (%)[y]	
		Unwounded	Wounded
C. gloeosporioides	F211117	25.0 ± 25.0	100 ± 0
C. truncatum	F211178	75.0 ± 25.0	100 ± 0
C. boninense	F210016	12.5 ± 12.5	100 ± 0
Control (ddH_2O)	-	0.0 ± 0.0	0 ± 0

[z] *Colletotrichum* isolates, F211178, F211117, and F210016, representing conidial types 1, 2, and 3, respectively, were used for inoculation tests on wounded and unwounded fruits of pitaya variety *Hylocereus undatus*. The experiment was conducted 2 times.
[y] Disease incidence (%) = (No. of infected sites)/(Total No. of inoculated sites) × 100%, values are mean ± standard error.

of Japan. Its conidia are characterized by with basal hilum and peripheral thickening at the tip of conidiogenous cell. However, the marked deviation among collected isolates were revealed by rDNA ITS phylogenetic analysis, also implicating composite of species complex. A multi-locus phylogenetic study conducted by Damm *et al*. (2012) using 7 genes: ITS, ACT, TUB2, CHS-1, GAPDH, HIS-3 (histoine-3) and calmodulin (CAL), with maximum parsimony and maximum likelihood methodologies, of the 86 isolates and other strains which previously recognized as *C. boninense* actually comprised of 18 clades. These clades were accepted as individual species and totally 17 species were included in the *C. boninense* species complex (Damm *et al*. 2012). Likewise, *Collectotrichum* species with curved conidia and dark setae were grouped in the *C. dematium* complex in general. Damm *et al*. (2009) performed a comprehensive exploration to determine the phylogenetic affiliation of the 97 isolates, including *C. dematium*, *C. lineola*, *C. truncatum* and other *Collectorrichum* species with curved conidia, using multilocus genes: ITS, ACT, TUB2, CHS-1, GAPDH, HIS-3. As a result, 20 clades resolved, four new species described: *C. anthrisci*, *C. liriopes*, *C. rusci* and *C. verruculosum*; two new combinations *C. spaethianum* and *C. tofieldiae* were made, and *C. truncatum* was epitypified; *C. capsici* was sanctioned as basionym of *C. truncatum*. In this paper, we considered *Colletotrichum* isolates F211117 and F210016, including other isolates possessing cylindrical conidial types 1 and 3 as *C. gloeosporioides* and *C. boninense*, whereas *Colletotrichum* isolates F211118 possessing curved conidial type 2 as *C. truncatum* listed in Table 2, respectively. However, multi-locus phylogenetic analysis would be useful for further discrimination of species identity within populations of the 124 isolates (Cai *et al*. 2009; Damm *et al*. 2009; Prihastuti *et al*. 2009; Damm *et al*. 2012; Weir *et al*. 2012).

Fruit anthracnose of *Hylocereus* spp. caused by *Colletotrichum* spp. was reported in several countries, including *C. gloeosporioides* in Malaysia (Masyahit *et al*. 2009) and Brazil (Takahashi *et al*. 2008), and *C. truncatum* in China (Guo *et al*. 2014). This study demonstrates that, in addition to *C. gloeosporioides* and *C. truncatum* reported in other countries and in Taiwan in this study, *C. boninense* is also recognized as a causal agent of fruit anthracnose of pitaya in Taiwan (Tables 1 and 2). Among the three *Colletotrichum* species, *C. gloeosporioides* is the most predominant and important postharvest pathogen of pitaya in Taiwan, followed by *C. truncatum*, and *C. boninense*.

The results of artificial inoculation tests indicate that wounding is a conducive factor for infection of *C. gloeosporioides*, *C. truncatum*, or *C. boninense* on pitaya fruits (Table 3). This explains why symptoms of pitaya anthracnose are frequently observed at the cutting sites of fruit stem or scales of pitaya fruits. It is of paramount importance to develop effective methods for protection of pitaya fruits from injury before and after harvest.

Colletotrichun gloeosporioides is a pathogen with wide host range including numerous economically important crops (Freeman *et al*. 1998; Perfect *et al*. 1999). Preliminary inoculation tests showed that *C. gloeosporioides* isolated from diseased fruits of pitaya also caused anthracnose on fruits of papaya (*Carica papaya*) and leaves of Chinese cabbage (*Brassica rapa chinensis*) and sweet pepper (*Capsicum annuum*) (Lin *et al*. unpublished data). On the other hand, *C. gloeosporioides* from diseased fruits of papaya, avocado (*Persea americana*), or grapes (*Vitis vinifera*) could cause symptoms of anthracnose on pitaya fruits by artificial inoculation (Lin *et al*. unpublished data). These results suggest that isolates of *C. gloeosporioides* from pitaya fruits and fruits of other crops are lack of host specificity.

The optimal temperature range for hyphal growth of *Colletotrichum* spp. from pitaya fruits is 28–32℃ (Fig. 4) which is similar to the temperature range for favorable infection of pitaya fruits in the orchards in Taiwan. Thus, severe

losses in marketable fruits may occur if pitaya fruits are produced at the temperature 28–32℃ in the orchard and stored at room temperature (28–32℃) after harvest. Since low temperature (below 8℃) is unfavorable for hyphal growth of *Colletotrichum* spp. (Fig. 4), it is recommended to store pitaya fruits at cooler temperatures (below 8℃) to minimize losses of harvested fruits during storage and transit period. As *Colletotrichum* spp. are known as latent infection pathogens (Baker *et al*. 1940; Cerkauskas 1988; Perfect *et al*. 1999), infection of pitaya fruits by the *Collectotrichum* pathogens may have already occurred in the field but quiescent and remained symptomless at harvest. The symptoms of anthracnose could develop rapidly if the infected fruits are stored at high temperature (28–32℃) and high humidity. Therefore, in addition to storing harvested fruits of pitaya at cooler temperature (below 8℃), practice for protection of pitaya fruits from infection of *Colletotrichum* spp. in the field such as bagging of young fruits or application of chemical fungicides at right time period may be effective in preventing pitaya fruits from infection by anthracnose pathogens.

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台灣新紀錄之引起採收後紅龍果炭疽病之 *Colletotrichum* spp.

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摘要

林筑蘋、安寶貞、黃鴻章、張捷婷、蔡志濃。2017。台灣新紀錄之引起採收後紅龍果炭疽病之 *Colletotrichum* spp.。台灣農業研究 66(3):171–183。

紅龍果 (白肉 *Hylocereus undatus*，紅肉 *H. polyrhizus* 及 *H. costaricensis*) 為台灣近 20 年來廣受栽培的果樹，依 2009 到 2013 的田間調查顯示，由 *Colletotrichum* spp. 引起的炭疽病為台灣紅龍果主要採收後病害，導致果實倉儲期縮短或降低商品價值，以及經濟價值損失。病斑自果實採收後室溫儲藏 5–10 d 間開始出現，表皮呈現褐色凹陷，嚴重者可能出現水浸狀腐爛。以組織分離罹病果實上的病原菌，並根據形態以及 ITS 區域序列及類緣分析，至少確定有 3 種 *Colletotrichum* spp.，包括 *C. gloeosporioides*、*C. truncatum* 與 *C. boninense* 可感染紅龍果。2009 到 2013 期間自罹染炭疽病之組織上分離各病原之分離率分別為 73.4、21.8 及 4.8%。近年來其他學者發現 *C. gloeosporioides* 與 *C. boninense* 個別為複合族群 (species complex)，族群內可再藉由多基因親源分析 (multi-locus phylogenetic analysis) 區分出多個新種 *Colletotrichum* spp.。為鑑定感染紅龍果之 *Colletotrichum* spp.，未來將再進行多基因親源分析。紅龍果炭疽病為台灣紅龍果重要之採收後病害，本篇在台灣為 *Colletotrichum* spp. 引起之紅龍果炭疽病之首篇報導。

關鍵詞：紅龍果、採後病害、炭疽病、*Colletotrichum* spp.。

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