台灣農業研究 (J. Taiwan Agric. Res.) 70(2):81–97 (2021)
DOI:10.6156/JTAR.202106_70(2).0001

Research Article

Avocado Branch Canker Disease Caused by *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* in Taiwan

Yu-Ping Liang[1], Chao-Jung Wu[1], Hui-Wen Tsai[2], and Hui-Fang Ni[3,*]

Abstract

Liang, Y. P., C. J. Wu, H. W. Tsai, and H. F. Ni. 2021. Avocado branch canker disease caused by *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* in Taiwan. J. Taiwan Agric. Res. 70(2):81–97.

Avocado branch canker disease has been reported in many avocado-growing countries, and is associated with several species in the Botryosphaeriaceae family. In Taiwan, the disease is widely distributed in the main avocado-producing regions, but the pathogens were still unidentified. Thirteen fungal isolates were obtained from necrotic avocado woody tissues from seven orchards located in different regions of Taiwan. Based on morphology and phylogenetic analyses of the internal transcribed spacer sequence and partial sequence of the translation elongation factor 1-α gene, the isolates were identified as either *Lasiodiplodia theobromae* or *Lasiodiplodia pseudotheobromae*. The optimal temperature for mycelial growth for both species was around 30℃. Both species were pathogenic to avocado stems and fruits, causing a whitish exudate at the inoculation site and necrosis on the stem, and black lesions on the fruit. To our knowledge, this is the first report characterizing avocado branch canker in Taiwan, which is different from temperate regions in major pathogenic species. The information obtained in this study will be helpful for understanding the epidemiology of the pathogens and establishing effective disease management strategies in avocado.

Key words: Avocado, Branch canker, *Lasiodiplodia theobromae*, *Lasiodiplodia pseudotheobromae*, Taiwan.

INTRODUCTION

Avocado (*Persea americana* Mill.) is cultivated worldwide in tropical and subtropical regions. It was introduced to Taiwan in the twentieth century, and production has gradually increased due to an increasing consumer demand for avocado in recent years. The major avocado production areas are concentrated in the southern cities of Taiwan, including Tainan and Chiayi. In 2019, the planted acreage was 930 hectares with an annual yield of 10,366 Mg (http://agrstat.coa.gov.tw/sdweb/public/inquiry/InquireAdvance.aspx). Instead of 'Hass', which is the most commercially popular avocado worldwide, the major cultivars planted in Taiwan are 'Hall', 'Choquette', and local cultivars derived mostly from open-pollinated West Indian varieties, such as 'Hung Shin Yuan', 'Tainung No.1 Tasty Red', 'Tainung No.2 Green Gold',

Received: November 19, 2020; Accepted: December 28, 2020.
* Corresponding author, e-mail: hfni@dns.caes.gov.tw
[1] Assistant Research Fellows, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[2] Contract Assistant Research Fellow, Department of Horticulture, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[3] Associate Research Fellow and Head, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.

'Zhongpu Green Skin', 'CAES 2', 'CAES 3', 'CAES 4', and 'Changan'. This is because the West Indian race and its hybrids with the Guatemalan race perform better in tropical areas (Ghosh 2000). However, most orchards are small (less than 2 hectares), and planted with a variety of cultivars with different flower types and harvest seasons ranging from June to February.

Avocado branch cankers have been reported in many avocado-growing countries, including the United States (McDonald & Eskalen 2011), Chile (Valencia *et al.* 2019), Italy (Guarnaccia *et al.* 2016), and Spain (Zea-Bonilla *et al.* 2007; Arjona-Girona *et al.* 2019). Previously published studies indicated that avocado branch canker could occur on twigs, branches, or trunks of avocado (McDonald & Eskalen 2011). Cankers are often associated with wounds from pruning, frost damage, mechanical damage, split bark from wind damage, and grafting. In addition, the incidence and severity of disease is greater in trees suffering from drought stress, nutrient deficiencies, waterlogging, temperature extremes, or damage by insects or other pathogens (Dann *et al.* 2013). The typical symptoms include dark, cracked, or sunken bark, which often exudes a reddish sap that dries to a whitish-beige powder; underneath the canker, the wood is reddish brown or brown (McDonald & Eskalen 2011; Auger *et al.* 2013; Dann *et al.* 2013). A cross section of a cankered branch might show a characteristic wedge-shaped discoloration extending to the xylem (McDonald & Eskalen 2011). Pathogen colonization can affect water and nutrient transport, and weaken the woody tissue, causing the affected branch to wilt rapidly (McDonald & Eskalen 2011; Auger *et al.* 2013; Eskalen *et al.* 2013).

Pathogens associated with avocado branch canker include *Neofusicoccum australe*, *Neofusicoccum luteum*, *Neofusicoccum parvum*, *Neofusicoccum nonquaesitum*, *Fusicoccum aesculi*, *Dothiorella iberica*, *Diaporthe foeniculacea*, *Diaporthe sterilis*, *Diplodia mutila*, *Diplodia pseudoseriata*, *Diplodia seriata* (McDonald & Eskalen 2011; Eskalen *et al.* 2013; Guarnaccia *et al.* 2016), and *Neocosmospora perseae* (Guarnaccia *et al.* 2018). Most of the reported branch canker pathogens are species in the Botryosphaeriaceae family, members of which are associated with a wide variety of woody hosts (Slippers & Wingfield 2007). On avocado, they are also the casual agents of stem-end rot on fruits (Twizeyimana *et al.* 2013; Guarnaccia *et al.* 2016; Valencia *et al.* 2019). In Taiwan, the major species causing avocado stem-end rot are *Lasiodiplodia theobromae*, *N. parvum*, *Neofusicoccum mangiferae*, and *F. aesculi* (Ni *et al.* 2011).

Avocado branch canker is a long-standing problem in Taiwan, but the pathogens have not yet been identified. Therefore, to provide information for establishing effective disease management strategies, the objectives of this study were to identify and characterize the pathogens associated with avocado branch canker in Taiwan by morphological examination and molecular analysis, and evaluate their pathogenicity.

MATERIALS AND METHODS

Field sampling and fungal isolation

Sampling was conducted in seven avocado orchards from 2017 to 2019. In each orchard, symptomatic branches were cut from trunks and transported to the laboratory. Small wood pieces were removed from the margins between the healthy and necrotic tissues. The samples were disinfected by immersion in 0.5% NaClO for 30 s, rinsed with sterile water, and then placed in Petri dishes containing 2% water agar. The plates were incubated at 25℃ for 1 to 4 d. Single hyphal tips were transferred to potato dextrose agar (PDA, Difco Inc., Detroit, MI, USA) to obtain a pure culture. All the isolates were stored in 1 mL of sterile water at two different temperatures, 10℃ and 25℃.

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

Mycelia from each isolate were collected from colonies cultivated on PDA and DNA was extracted using QuickExtract™ Plant

DNA Extraction Solution (Epicenter, Madison, WI, USA). The internal transcribed spacer 1 (ITS1)-5.8S-ITS2 region of ribosomal DNA (referred to as the ITS) and partial sequence of the translation elongation factor 1-α (TEF1-α) were amplified using primers ITS4 and ITS5 (White *et al.* 1990), and EF1F and EF2R (Jacobs *et al.* 2004), respectively. For isolate AZP69, sequence of TEF1-α was amplified using primers EF688F and EF1251R (Alves *et al.* 2008). PCR was performed in a 25-μL reaction mixture containing 5 μL of Fast-Run™ Taq Master Mix 5× (Protech Technology Enterprise Co., Ltd., Taipei, Taiwan), 18 μL of ddH_2O, 0.5 μL of each primer (10 pmole μL^{-1}), and 1 μL of DNA template (50 ng μL^{-1}). The PCR conditions for the primers ITS4 and ITS5 were 4 min of initial denaturation at 94℃, followed by 35 cycles of 30 s of denaturation at 94℃, 30 s of annealing at 52℃, and 30 s of extension at 72℃, and a final extension of 4 min at 72℃. For the primers EF1F, EF2R, EF688F and EF1251R, the PCR conditions were 4 min of initial denaturation at 94℃, followed by 35 cycles of 30 s of denaturation at 94℃, 30 s of annealing at 55℃, and 45 s of extension at 72℃, and a final extension of 5 min at 72℃. The PCR product was purified and sequenced by Tri-I Biotech, Inc. (Taipei, Taiwan).

Phylogenetic analyses

Bayesian inference was used to construct phylogenetic trees. The ITS and TEF1-α nuclear gene regions were used in the analysis. Sequences of 56 *Lasiodiplodia* isolates from the GenBank database were included in the tree (Table 1). Multiple sequence alignment of each gene was conducted using ClustalX v. 2.1 (Larkin *et al.* 2007), and these alignments were concatenated using SequenceMatrix v. 2.1.10 (Vaidya *et al.* 2011). Modeltest (Darriba *et al.* 2012) was used to choose the best-fit DNA substitution model under the Bayesian information criterion (BIC). The DNA substitution model used for ITS and TEF1-α were K80 + I and GTR + G, respectively. Phylogenetic tree construction was conducted with MrBayes v. 3.2.6 (Ronquist *et al.* 2012). The analysis was run twice for 1×10^7 generations, and samples were taken from the posterior every 1,000 generations. The first 25% of generations were discarded as burn-in. The tree is rooted with *Barriopsis fusca* CBS174.26. The tree and matrices were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S27172).

Morphological characterization

Colony morphology was characterized by growing isolates on PDA for 3 d at 25℃ in darkness. Production of pycnidia and conidia was induced by placing mycelial plugs on 2% (w/v) water agar (Merck, Darmstadt, Germany) supplemented with autoclaved horsetail stem (*Casuarina equisetifolia* L.) (Ni *et al.* 2011; Kee *et al.* 2017) and incubating for over 21 d at 25℃ under black light (TL-D 18W BLB, Philips, Amsterdam, Netherlands). Morphological characteristics (e.g., color, shape, and septation of conidia and paraphyses) were observed with a Nikon 80i microscope (Tokyo, Japan), and pictures were captured with a Progres Gryphax camera (Jenoptik, Jena, Germany). The length and width of 50 conidia were measured.

Effect of temperature on mycelial growth

Mycelial discs (5 mm in diameter) of thirteen isolates obtained from necrotic avocado wood tissues (Table 2) were transferred to PDA in Petri dishes (9 cm in diameter) and incubated at 10, 15, 20, 25, 30 and 35℃ in darkness. There were six replicates for each temperature. The colony diameter was measured at 24 h.

Pathogenicity test on stems

One-year-old grafted 'Hall' avocado plants were kept in a greenhouse (ambient temperature 25–45℃). Healthy stems about 1 cm diameter were selected for inoculation. The surface of the stem was disinfected with 75% ethanol. A piece of bark (5 mm in diameter) was removed from the stem with a sterilized cork borer. *L. theobromae* ADN21 and *Lasiodiplodia pseudotheobromae* AZC38 were selected for the pathogenicity test as they were isolated from the two

Table 1. GenBank accession numbers for DNA sequences of *Lasiodiplodia* spp. used in the phylogenetic analyses.

		GenBank accession number[z,y]	
Species	Isolate	ITS	TEF1-α
Barriopsis fusca	CBS 174.26	KF766149	KF766395
Lasiodiplodia brasiliense	COAD 1784	KP244693	KP308469
L. brasiliense	COAD 1786	KP244696	KP308471
L. brasiliense	COAD 1787	KP244695	KP308470
L. brasiliense	CDA 431	KP244694	KP308472
L. brasiliense	CMM4015	JX464063	JX464049
Lasiodiplodia crassispora	CBS110492	EF622086	EF622066
L. crassispora	CMW13488	FJ888465	FJ888452
Lasiodiplodia egyptiacae	BOT29	JN814401	JN814428
L. egyptiacae	CBS130992	JN814397	JN814424
L. egyptiacae	COAD 1791	KP244687	KP308462
Lasiodiplodia euphorbiicola	CMM3652	KF234554	KF226715
L. euphorbiicola	CMM3609	KF234543	KF226689
Lasiodiplodia exigua	CBS137785	KJ638317	KJ638336
L. exigua	BL185	KJ638319	KJ638338
Lasiodiplodia gilanensis	IRAN1523C	GU945351	GU945342
L. gilanensis	IRAN1501C	GU945352	GU945341
Lasiodiplodia gonubiensis	CBS115812	DQ458892	DQ458877
Lasiodiplodia hormozganensis	IRAN1500C	GU945355	GU945343
L. hormozganensis	IRAN1498C	GU945356	GU945344
Lasiodiplodia iraniensis	IRAN 921C	GU945346	GU945334
L. iraniensis	IRAN 1519C	GU945350	GU945338
Lasiodiplodia jatrophicola	CMM3610	KF234544	KF226690
Lasiodiplodia macrospora	CMM3833	KF234557	KF226718
L. mahajangana	CMW27801	FJ900595	FJ900641
L. mahajangana	CMW27820	FJ900597	FJ900643
L. missouriana	UCD2193M	HQ288225	HQ288267
L. missouriana	UCD2199MO	HQ288226	HQ288268
Lasiodiplodia parva	CBS456.78	EF622083	EF622063
L. parva	CBS494.78	EF622084	EF622064
L. parva	CBS495.78	EF622085	EF622065
Lasiodiplodia plurivora	STEU-5803	EF445362	EF445395
Lasiodiplodia pseudotheobromae	COAD 1785	KP244690	KP308464
L. pseudotheobromae	CBS116459	EF622077	EF622057
L. pseudotheobromae	CBS116460	EF622078	EF622058
L. pseudotheobromae	CMM3887	KF234559	KF226722
L. pseudotheobromae	CBS 447.62	EF622081	EF622060

Table 1. GenBank accession numbers for DNA sequences of *Lasiodiplodia* spp. used in the phylogenetic analyses. (continued)

Species	Isolate	GenBank accession number[z,y]	
		ITS	TEF1-α
L. pseudotheobromae	**AZC38**	**MN911394**	**MN921243**
L. pseudotheobromae	**ADN23**	**MN911393**	**MN921242**
L. pseudotheobromae	**AZC12**	**MN911392**	**MN921241**
L. pseudotheobromae	**AFL8**	**MT093794**	**MT086519**
Lasiodiplodia pyriformis	CBS121770	EU101307	EU101352
L. pyriformis	CBS121771	EU101308	EU101353
Lasiodiplodia subglobosa	CMM3872	KF234558	KF226721
L. subglobosa	CMM4046	KF234560	KF226723
Lasiodiplodia theobromae	CBS111530	EF622074	EF622054
L. theobromae	CBS 164.96	AY640255	AY640258
L. theobromae	CBS 124.13	DQ458890	DQ458875
L. theobromae	CBS 339.90	EF622072	EF622052
L. theobromae	CMW9074	AY236952	AY236901
L. theobromae	COAD 1788	KP244698	KP308476
L. theobromae	COAD 1789	KP244700	KP308474
L. theobromae	CDA 425	KP244697	KP308475
L. theobromae	CDA 444	KP244699	KP308477
L. theobromae	CDA 450	KP244688	KP308478
L. theobromae	CDA 455	KP244689	KP308463
L. theobromae	CDA 467	KP244702	KP308473
L. theobromae	**ADN21**	**MN911388**	**MN921237**
L. theobromae	**ACY1**	**MN911389**	**MN921238**
L. theobromae	**ACY4**	**MN911390**	**MN921239**
L. theobromae	**AZC42**	**MN911391**	**MN921240**
L. theobromae	**AZP58**	**MT093789**	**MT086514**
L. theobromae	**AZP69**	**MT093790**	**MT086515**
L. theobromae	**AFL1**	**MT093791**	**MT086516**
L. theobromae	**ASS25**	**MT093792**	**MT086517**
L. theobromae	**ASS28**	**MT093793**	**MT086518**
Lasiodiplodia viticola	UCD2604MO	HQ288228	HQ288270
L. viticola	UCD2553AR	HQ288227	HQ288269
Lasiodiplodia venezuelensis	CBS118739	DQ103547	DQ103568
L. venezuelensis	CMW13512	DQ103549	DQ103570

[z] ITS: internal transcribed spacer regions 1 and 2, including the 5.8S ribosomal ribonucleic acid (RNA) gene; and TEF1-α: translation elongation factor 1-α.

[y] Isolates in boldface were obtained in this study.

Table 2. Conidial measurements of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* obtained from avocado in Taiwan and those obtained in previous studies

Species	Isolate	Location	Conidial size (μm)			Source
			Length[z]	Width[y]	L/W[x]	
L. theobromae	ADN21	Danei, Tainan	20.5-24.6-28.3	12.5-14.0-15.5	1.8	This study
	ACY1	Chiayi	19.4-24.0-28.8	10.7-13.9-17.9	1.8	This study
	ACY4	Chiayi	19.1-22.2-24.5	12.0-13.4-15.5	1.7	This study
	AZC42	Zhuqi, Chiayi	20.2-22.6-25.3	11.9-13.5-14.5	1.7	This study
	AZP58	Zhongpu, Chiayi	19.0-23.6-27.2	11.1-13.4-15.5	1.8	This study
	AZP69	Zhongpu, Chiayi	22.1-24.7-27.3	10.8-13.3-14.9	1.9	This study
	AFL1	Fanlu, Chiayi	21.2-24.4-28.4	12.3-13.8-15.7	1.8	This study
	ASS25	Shanshang, Tainan	19.1-21.6-24.8	11.1-12.3-14.1	1.8	This study
	ASS28	Shanshang, Tainan	21.3-24.2-27.9	11.5-13.2-15.3	1.8	This study
	-	-	21.0-26.2-31.0	13.0-14.2-15.0	1.9	Alves *et al.* (2008)
			22.4-24.2	12.9-14.3	1.8	Abdollahzadeh *et al.* (2010)
			24.49-27.49	13.30-14.79	1.8	Marques *et al.* (2013)
	CBS 164.96	-	19.0-26.2-32.5	12.0-14.2-18.5	1.9	Phillips *et al.* (2013)
			20.7-22.7	11.7-14.1	1.8	Netto *et al.* (2014)
	-	-	24.5-28.2	13.3-15.1	1.8	Correia *et al.* (2016)
	-	-	18.5-30.5	12-18	-	Rosado *et al.* (2016)
	PALUC449F	-	16.7-20.9-26.2	11.1-12.7-14.5	1.7	Valencia *et al.* (2019)
L. pseudotheobromae	AZC38	Zhuqi, Chiayi	21.1-25.8-32.7	12.4-14.4-18.1	1.8	This study
	ADN23	Danei, Tainan	22.4-24.9-30.3	10.1-12.9-14.4	2.0	This study
	AZC12	Zhuqi, Chiayi	19.3-22.5-25.6	11.2-13.6-15.5	1.7	This study
	AFL8	Fanlu, Chiayi	22.9-26.4-30.0	11.9-16.3-19.3	1.6	This study
			21.7-26.3	13.4-14.8	1.7	Abdollahzadeh *et al.* (2010)
			25.07-28.23	13.4-15.6	1.8	Marques *et al.* (2013)
			21.2-25.8	12.5-13.9	1.8	Netto *et al.* (2014)
	-	-	23.5-28.0-32.0	14-16-18	1.7	Alves *et al.* (2008)
	-	-	25.3-29.6	14.7-16.8	1.8	Correia et al. (2016)
	-	-	25-32	14-18	-	Rosado et al. (2016)

[z] Minimum length-average length-maximum length (or minimum length-maximum length when the average was not available).
[y] Minimum width-average width-maximum width (or minimum width-maximum width when the average was not available).
[x] L/W = average length/average width.

major avocado production areas in Taiwan. A 2-day-old mycelial plug (5 mm in diameter) was placed in the wound with the mycelial side facing the wound. Control plants were treated with sterile PDA discs (5 mm in diameter). The wound was sealed with parafilm to prevent dehydration, which was unwrapped at 2 wk after inoculation. The experimental design was completely randomized with 10 replicates for each treatment. The lengths of the external necrotic lesions were measured weekly. After 4 wk, the stems were cut and the lengths of the lesions that developed inside the stem were measured. Pathogens were re-isolated from lesions and re-identified by analysis of colony morphology and ITS sequences to fulfill Koch's postulates. The experiment was repeated twice.

Pathogenicity test on fruits

Two local cultivars, 'Changan' and 'Zhongpu Green Skin', were chosen for pathogenicity test because their fruits remain green-skinned after ripening, making lesion observation more convenient. The fruits were harvested at maturity and surface disinfected with 75% ethanol. Two-day-old mycelial plugs (5 mm in diameter) of *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 were placed on the fruits with the mycelial side facing the fruits. Control fruits were treated with sterile PDA discs (5 mm in diameter). The fruits were either unwounded or wounded with a flamed-sterilized needle (5 mm in depth). There were 5 replicates for each treatment. The fruits were kept at 25℃ in 100% relative humidity for the first 2 d after inoculation, and then the relative humidity were reduced to 65% for the rest of the trial. The diameters of the necrotic lesions on fruits were measured at 3 and 5 d after inoculation, and the data were subjected to one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test using SAS Enterprise Guide version 7.15. Pathogens were re-isolated from lesions and re-identified by analysis of colony morphology and TEF1-α sequences.

RESULTS

Field symptoms and fungi isolation

A total of 13 isolates were recovered from symptomatic avocado branches from seven different orchards (Table 2). The symptoms included dieback of affected branches (Fig. 1A) and internal browning visible in the cross section of a wilted branch (Fig. 1B). Darkening of bark with extrusion of reddish sap also occurred, and the wood underneath the bark turned reddish brown (Figs. 1C–1D). In addition, there was evidence

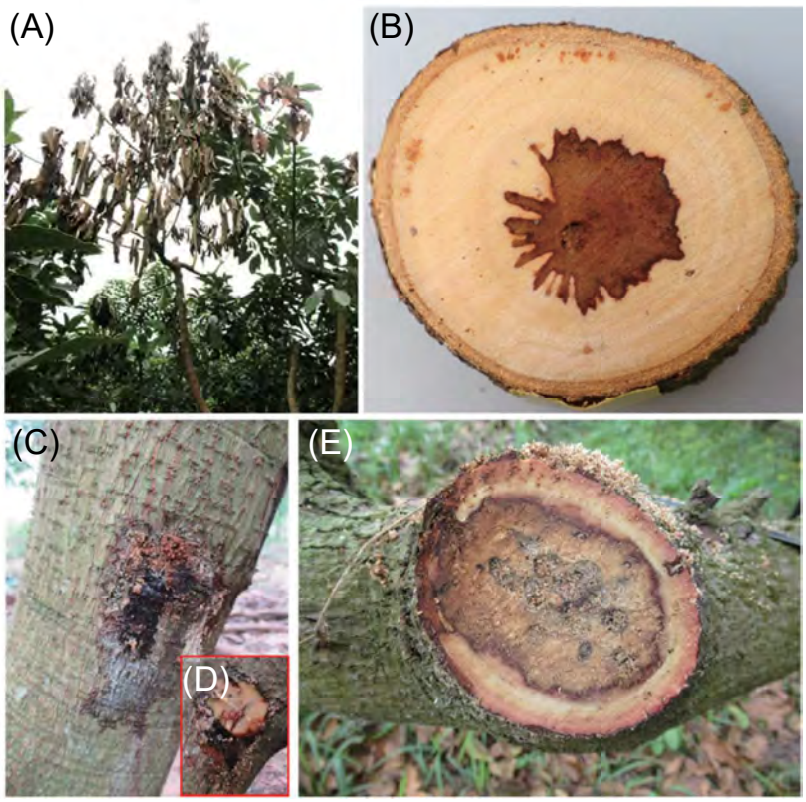

Fig. 1. Symptoms of avocado branch canker in the field. (A) Dieback of an affected branch; (B) internal browning in a wilted branch; (C) discolored bark with reddish sap; (D) reddish brown wood underneath the bark; and (E) browning and decay visible in the cross section of an old pruning wound.

that infection could also start from a mechanical injury, such as a pruning cut. For example, when pruning was not done properly, i.e., cutting too far from the branch collar and leaving a section of dead limb on the tree, it appears that the pruning wounds did not heal properly and infection occurred, making the inner tissues turn brown and decay (Fig. 1E).

Phylogenetic analysis

DNA sequences of the two gene regions were obtained from 70 isolates included in this study and concatenated to form a supermatrix of 1,347 bps. The result revealed the thirteen avocado isolates in this study belonged to *L. theobromae* or *L. pseudotheobromae* (Fig. 2). Four isolates, AND23, AFL8, AZC12 and AZC38, were under the clade *L. pseudotheobromae* with a high Bayesian posterior probability (BPP) value of 100%. Nine *L. theobromae* isolates (ACY1, ACY4, AFL1, ADN21, ASS25, ASS28, AZC42, AZP58 and AZP69) were under *L. theobromae* clade.

Morphological characterization

The conidial size of each isolate is shown in Table 2. *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 were selected for further morphological characterization, as they were also tested for pathogenicity. Colonies of *L. theobromae* ADN21 grown on PDA were greyish white on the upper surface with fluffy aerial mycelia, and the lower surface was greyish white initially but became dark green after 3 d (Figs. 3A–3B). Paraphyses were hyaline, cylindrical, and aseptate. Conidia were subovoid to ellipsoid. Immature conidia were hyaline and aseptate, and mature conidia were dark brown and 1-septate, measuring 24.6 μm × 14.0 μm on average (n = 50) (Table 2, Figs. 3C–3E). Colonies of *L. pseudotheobromae* AZC38 grown on PDA were also grey-white on the upper surface with fluffy aerial mycelia, but the lower surface remained greyish white after 3 d (Figs. 4A–4B). Paraphyses were hyaline, cylindrical, and aseptate. Conidia were subovoid to ellipsoid. Immature conidia were hyaline and aseptate, and mature conidia were dark brown and 1-septate, measuring 25.8 μm × 14.4 μm on average (n = 50) (Table 2, Figs. 4C–4E).

Effect of temperature on mycelial growth

All 13 isolates of *L. theobromae* and *L. pseudotheobromae* could grow in the range of 10–35℃. *L. theobromae* grew slightly faster than *L. pseudotheobromae* on average, but not all isolates of *L. theobromae* grew faster than *L. pseudotheobromae* isolates (Fig. 5). Optimal mycelial growth for both species was observed at 30℃; the average colony diameters of *L. theobromae* and *L. pseudotheobromae* at this temperature were 58.9 mm and 53.8 mm, respectively.

Pathogenicity test on stems

Both *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 were pathogenic to 'Hall' avocado. After inoculation with *L. theobromae* ADN21 or *L. pseudotheobromae* AZC38, excretion of a white powder and an external black lesion were observed at the inoculation site of every stem, while the inoculation site in the control treatment healed by the end of trial (Figs. 6A–6C). The external black lesions at the *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 inoculation sites expanded by 0.79 cm and 1.10 cm, respectively, from week 2 to week 4 (Table 3). Underneath the lesions, the tissues turned brown along the vascular bundle, and the internal lesion lengths were longer the external lesions (Figs. 6E–6F). The average internal lesion lengths were 9.98 cm and 8.79 cm for *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38, respectively, at 4 wk after inoculation (Table 3). Both isolates were successfully re-isolated from necrotic tissues and re-identified based on cultural features and ITS sequences, while no *Lasiodiplodia* spp. were isolated from the control-treated tissues.

Pathogenicity test on fruits

Both *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 caused black lesions on avocado fruits. The external lesions were visible at 2 d after inoculation, and continued becoming larger. Underneath the lesions, the pulp turned soft, water-soaked, and black. In wounded in-

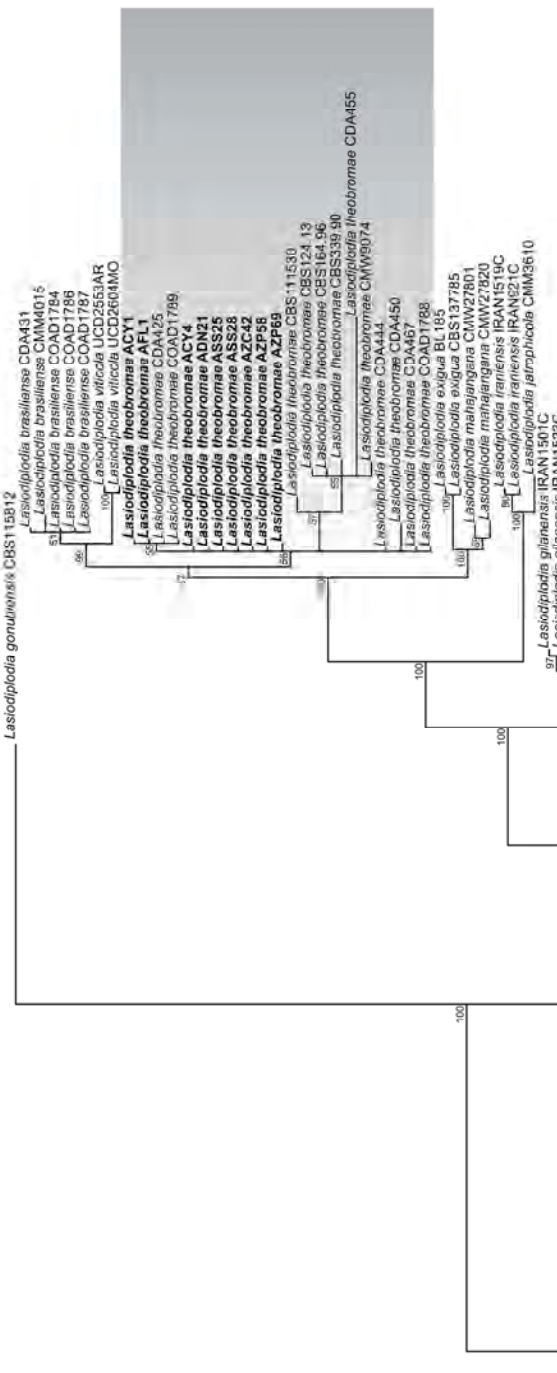

Fig. 2. Bayesian phylogenetic trees of *Lasiodiplodia* isolates from avocado in Taiwan and from GenBank database. The phylogenetic tree was built using concatenated sequences of partial internal transcribed spacer and translational elongation factor 1-α. The Bayesian posterior probabilities are indicated next to the nodes. The tree is rooted by *Barriopsis fusca* CBS174.26. Isolates from this study are emphasized in bold.

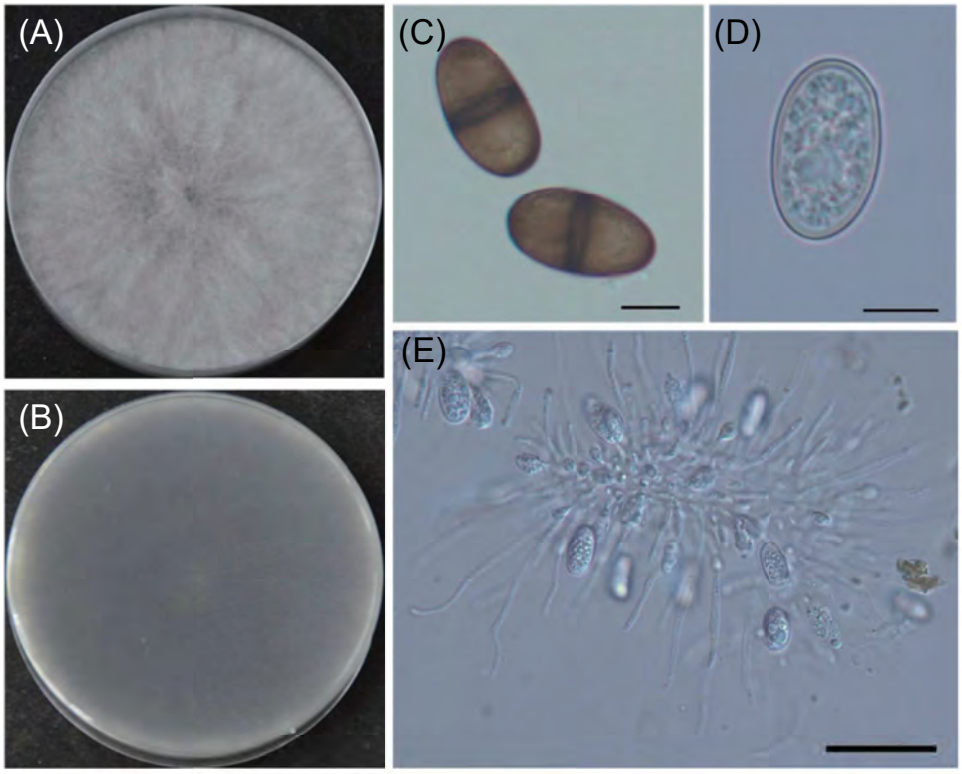

Fig. 3. Morphological characteristics of *Lasiodiplodia theobromae* ADN21. (A–B) Colony morphology on potato dextrose agar after 3 days at 25℃. (A) Top view; (B) bottom view; (C) mature conidia; (D) immature conidia; and (E) conidiogenous layer with conidiogenous cells and paraphyses. Scale bars: (C) and (D) = 10 μm; (E) = 50 μm.

oculation, both isolates caused lesions on both 'Changan' and 'Zhongpu Green Skin' fruits. At 3 d post inoculation, the average lesion diameter caused by *L. pseudotheobromae* AZC38 on 'Changan' fruits was 3.54 cm, which was significantly larger than the lesion caused by *L. theobromae* ADN21 (1.47 cm), while there was no significant difference between them on 'Zhongpu Green Skin' fruits. At 5 d post inoculation, both species caused lesions greater than 6 cm in diameter on both 'Changan' and 'Zhongpu Green Skin' fruits (Table 4). In unwounded inoculation, *L. pseudotheobromae* AZC38 caused lesions on both cultivars, while *L. theobromae* ADN21 only caused lesions on two 'Zhongpu Green Skin' fruits. Both isolates were successfully re-isolated from necrotic pulps and re-identified based on cultural features and TEF1-α sequences. All control fruits remained symptomless at treated sites, and no fungi were re-isolated.

DISCUSSION

Avocado branch canker is widely distributed in many avocado production areas in Taiwan. In this study, *L. theobromae* and *L. pseudotheobromae* were identified from symptomatic avocado branches. To our knowledge, this is the first report characterizing avocado branch canker in Taiwan.

The morphological characteristics of *L. theobromae* and *L. pseudotheobromae* isolates examined in this study generally corresponded to those reported in published studies (Alves *et al.* 2008; Phillips *et al.* 2013; Correia *et al.* 2016; Rosado *et al.* 2016; Valencia *et al.* 2019), although the conidial sizes were slightly smaller. The possible reasons might include different culture methods and differences between isolates. The morphology of *L. pseudotheobromae* is similar to that of *L. theobromae*. In fact, *L. pseudotheobromae* was previously considered

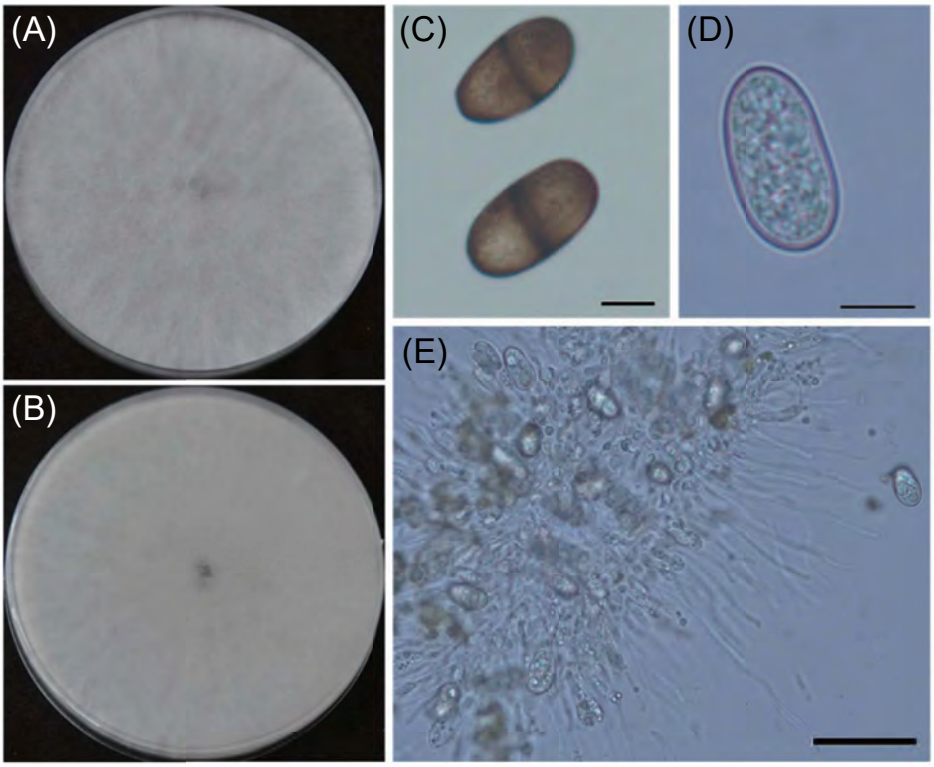

Fig. 4. Morphological characteristics of *Lasiodiplodia pseudotheobromae* AZC38. (A–B) Colony morphology on potato dextrose agar after 3 days at 25℃ (A, Top view; B, Bottom view); (C) mature conidia; (D) immature conidia; and (E) conidiogenous layer with conidiogenous cells and paraphyses. Scale bars: (C–D) = 10 μm; (E) = 50 μm.

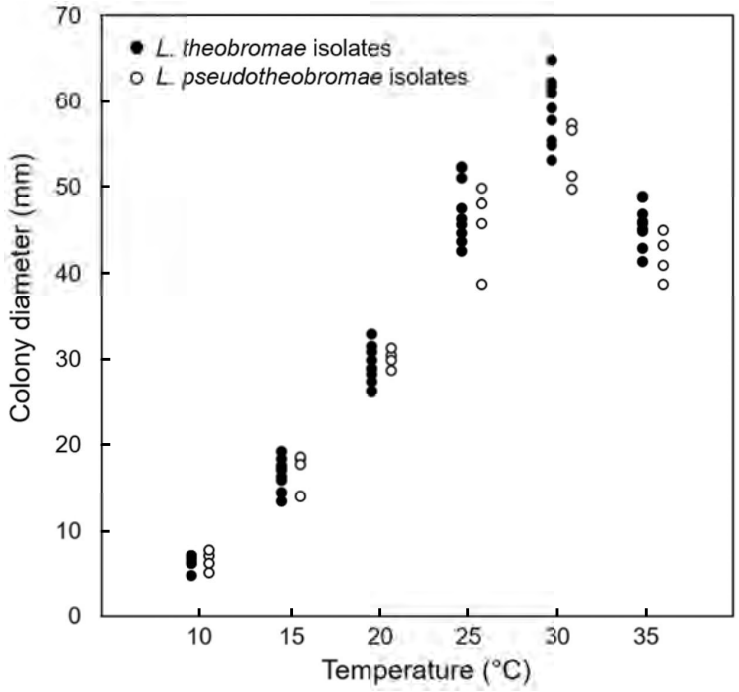

Fig. 5. Effect of temperature on mycelial growth of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* isolated in this study. Colony diameter (mm) was measured at 24 h after inoculation on potato dextrose agar. Each dot represents the average colony diameter for each isolate from six replicates.

to be *L. theobromae* until Alves *et al.* (2008) combined morphological and phylogenetic analyses to distinguish these species. According to Alves *et al.* (2008), *L. pseudotheobromae* differs from *L. theobromae* in its conidia, which are larger and more ellipsoid and do not taper as strongly toward the truncate base; in addition, *L. pseudotheobromae* can grow at 10℃. However, according to our study and previous studies, conidial shape ranges from subovoid to ellipsoid for all isolates, and conidial size also varies between different isolates. As for the differences in growth at low temperature, all isolates of both species in the present study could grow at 10℃, which is consistent with the findings of Abdollahzadeh *et al.* (2010), Marques *et al.* (2013) and Netto *et al.* (2014).

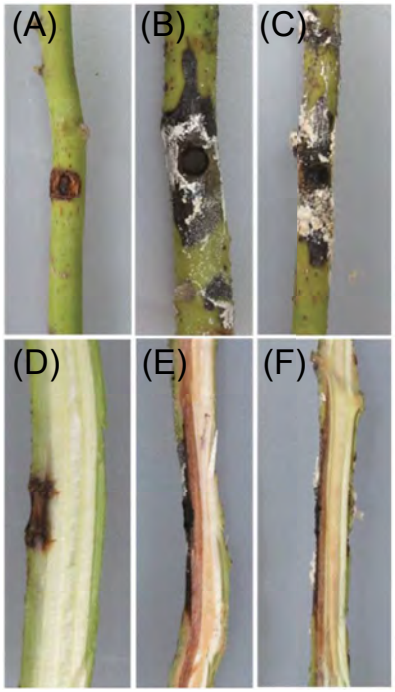

Fig. 6. (A–C) External lesions and (D–F) internal discoloration that developed 4 wk after artificial inoculation on 'Hall' avocado stems. (A, D) Control; (B, E) *Lasiodiplodia theobromae* ADN21; and (C, F) *Lasiodiplodia pseudotheobromae* AZC38.

Because morphological and culture characteristics might not be sufficient to distinguish closely related species of *Lasiodiplodia*, it is necessary to analyze sequences from multiple loci to identify *Lasiodiplodia* spp. Thirteen isolates obtained from different avocado growing areas in Taiwan were included in the phylogenetic analyses. All of them were identified as either *L. theobromae* or *L. pseudotheobromae* based on ITS and TEF-1α sequences.

The pathogenicity test on stems in this study showed that *L. theobromae* and *L. pseudotheobromae* are pathogenic to the avocado 'Hall' cultivar. Under the conditions used in the test, symptoms developed rapidly and lesions were visible after 2 wk of inoculation. Avocado branch canker has been reported to be associated with several fungal species in different countries, most of which are members of the Botryosphaeriaceae family, including *L. pseudotheobromae*, *N. austral*, *N. luteum*, *N. parvum*, *N. nonquaesitum*, *F. aesculi*, *D. iberica*, *Dip. mutila*, *Dip. pseudoseriata*, and *Dip. seriata* (McDonald & Eskalen 2011; Eskalen *et al.* 2013; Trakunyingcharoen *et al.* 2015; Guarnaccia *et al.* 2016). Interestingly, *L. theobromae* was not reported as a major pathogen in most previous studies investigating pathogens of avocado branch canker or

Table 3. Pathogenicity test of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* on 'Hall' avocado stem.

	Lesion length (M ± SD, cm)[z]				
Isolate	2 wk[y]	3 wk[y]	4 wk[y]	4 wk[x]	Incidence[w]
L. theobromae ADN21	3.60 ± 1.23	4.19 ± 1.71	4.39 ± 1.77	9.98 ± 4.03	10/10
L. pseudotheobromae AZC38	5.29 ± 3.03	6.24 ± 4.02	6.39 ± 4.00	8.79 ± 3.79	10/10
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.38	0/10

[z] Mean (M) and standard deviation (SD) are derived from 10 replicates.
[y] External lesions at weeks 2, 3 and 4.
[x] Internal lesions at 4 wk.
[w] Incidence = number of stems with external lesions/number of treated stems.

Table 4. Pathogenicity test of *Lasiodiplodia theobromae* ADN21 and *Lasiodiplodia pseudotheobromae* AZC38 on avocado fruits.

		Lesion diameter (M ± SD, cm)[z]					
		'Changan'			'Zhongpu Green Skin'		
Isolate	Wound[y]	3 d	5 d	Incidence[x]	3 d	5 d	Incidence[x]
L. theobromae ADN21	+	1.47 ± 1.08 b	6.24 ± 1.95 a	5/5	2.97 ± 0.95 a	7.45 ± 1.76 a	5/5
	−	0.00 ± 0.00 c	0.00 ± 0.00 c	0/5	0.44 ± 0.64 b	1.35 ± 2.09 b	2/5
L. pseudotheobromae AZC38	+	3.54 ± 0.63 a	7.83 ± 0.99 a	5/5	3.20 ± 0.44 a	8.00± 1.52 a	5/5
	−	1.42 ± 1.04 b	4.09 ± 2.37 b	4/5	2.51 ± 0.82 a	6.66 ±1.15 a	5/5
Control	+	0.00 ± 0.00 c	0.00 ± 0.00 c	0/5	0.00 ± 0.00 b	0.00 ± 0.00 b	0/5
	−	0.00 ± 0.00 c	0.00 ± 0.00 c	0/5	0.00 ± 0.00 b	0.00 ± 0.00 b	0/5

[z] Mean (M) and standard deviation (SD) are derived from 5 replicates. Means in the same column followed by the same letter are not significantly different according to Fisher's least significant difference (LSD) test ($P = 0.05$).
[y] "+" means wounded inoculation, while "−" means unwounded inoculation.
[x] Incidence = number of symptomatic fruits/number of treated fruits.

dieback (McDonald & Eskalen 2011; Eskalen *et al.* 2013; Guarnaccia *et al.* 2016), and seemed to be a minor avocado branch canker pathogen (Arjona-Girona *et al.* 2019) or more closely associated with stem-end rot (Valencia *et al.* 2019). *L. pseudotheobromae* has only been demonstrated to cause avocado stem canker in Thailand (Trakunyingcharoen *et al.* 2015).

The tropical climate of Taiwan might be one possible reason for the differences in pathogen species between Taiwan and most other countries. First of all, the avocado cultivars planted in Taiwan are mostly the West Indian race or West Indian × Guatemalan hybrids because they perform better in tropical areas, while major avocado production countries mostly plant 'Hass', a Guatemalan × Mexican hybrid. Similar to Taiwan, *L. pseudotheobromae* was also demonstrated to cause stem canker on avocado in Thailand (Trakunyingcharoen *et al.* 2015), where the most common avocados planted are West Indian × Guatemalan hybrids (Babpraserth & Subhadrabandhu 2000). Whether the virulence and distribution of Botryosphaeriaceae spp. on avocado stems varies among different cultivars requires further studies.

Secondly, the higher temperature in Taiwan might also result in the difference in pathogenic species between Taiwan and other countries. Previous studies indicated that climatic factors might not explain the distribution of Botryosphaeriaceae spp. on avocado because the same species could be detected in avocado orchards located in different climatic zones (McDonald & Eskalen 2011; Valencia *et al.* 2019). However, a phylogeographic study on the distribution of Botryosphaeriaceae in Australia indicated that although some species had a wide distribution across tropical and temperate regions, most *Lasiodiplodia* spp. were more common in tropical regions, while *Diplodia*, *Dothiorella* and most *Neofusicoccum* spp. were more common in temperate regions (Burgess *et al.* 2019). In addition, the optimal growth temperature of *Diplodia* spp. and *Neofusicoccum* spp. was around 25℃ (Valencia *et al.* 2019), while it was found to be around 30℃ for both *L. theobromae* and *L. pseudotheobromae* in this study and previously published studies (Netto *et al.* 2014; Valencia *et al.* 2019).

The annual average temperature in Tainan City, the major avocado production area in Taiwan, was around 25℃ in 2018, with a high temperature of 33℃ and low temperature of 14℃ (Central Weather Bureau 2018). The average temperature is clearly higher than that in temperate avocado production countries. For example, the average temperature of avocado production areas is around 14℃ in Chile (Barros & Sanchez 1992) and 13–18℃ in California (McDonald & Eskalen 2011). Therefore, it is possible that the warmer tropical climate in Taiwan might favor

the growth of *L. theobromae* and *L. pseudotheobromae*, resulting in the difference in pathogenic species compared with other countries. Similarly, *L pseudotheobromae* was also demonstrated to cause stem canker on avocado in Thailand (Trakunyingcharoen *et al.* 2015), which is also a tropical area. Further investigation of the fungal species associated with avocado branch canker in tropical regions would be helpful for understanding the epidemiology of Botryosphaeriaceae species on avocado and developing avocado disease management strategies in tropical regions.

The pathogenicity test on fruits in this study showed that *L. pseudotheobromae* caused lesions on both wounded and unwounded inoculated fruits, while *L. theobromae* only caused severe symptoms on wounded fruits. Though previous studies had demonstrated that both *L. theobromae* and *L. pseudotheobromae* were pathogenic to avocado fruits (Ni *et al.* 2011; Trakunyingcharoen *et al.* 2015; Valencia *et al.* 2019), this study further indicated that *L. pseudotheobromae* could invade avocado fruits without a wound. Therefore, *L. pseudotheobromae* might cause postharvest fruit rot not only at stem-ends, but also at other fruit parts.

As avocado branch canker is a major threat to avocado production worldwide, it is crucial to develop effective control methods. The results of this study suggest that the fungal pathogens associated with avocado branch canker might differ between tropical and temperate regions, indicating that in the future it is important to study the epidemiology of the pathogens and develop effective management strategies.

ACKNOWLEDGEMENTS

We thank the Council of Agriculture, Executive Yuan, Taiwan (ROC) for funding this research; Ms. S. L. Hsu and S. Y. Lai for their assistance in fungal pathogen isolation, temperature effect tests, and sequence analysis; and producers who allowed us to sample from their orchards.

REFERENCES

Abdollahzadeh, J., A. Javadi, E. M. Goltapeh, R. Zare, and A. J. L. Phillips. 2010. Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. Persoonia 25:1–10. doi:10.3767/003158510X524150

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

Arjona-Girona, I., D. Ruano-Rosa, and C. J. López-Herrera. 2019. Identification, pathogenicity and distribution of the causal agents of dieback in avocado orchards in Spain. Span. J. Agric. Res. 17:e1003. doi:10.5424/sjar/2019171-13561

Auger, J., F. Palma, I. Pérez, and M. Esterio. 2013. First report of *Neofusicoccum australe* (*Botryosphaeria australis*), as a branch dieback pathogen of avocado trees in Chile. Plant Dis. 97:842. doi:10.1094/PDIS-10-12-0980-PDN

Babpraserth, C. and S. Subhadrabandhu. 2000. Avocado production in Thailand. p.57–64. *in*: Avocado Production in Asia and the Pacific. (Papademetriou, M. K., ed.) Food and Agriculture Organization of the United Nations and Regional Office for Asia and the Pacific. Bangkok, Thailand. 72 pp.

Barros, R. and L. Sanchez. 1992. The Chilean avocado industry. p.639–642. *in*: Proceedings of the Second World Avocado Congress: The Shape of Things to Come. April 21–26, 1991. Orange, CA, USA. California Avocado Society, Ventura, CA.

Burgess, T. I., Y. P. Tan, J. Garnas, J. Edwards, K. A. Scarlett, L. A. Shuttleworth, R. Daniel, E. K. Dann, L. E. Parkinson, Q. Dinh, R. G. Shivas, and F. Jami. 2019. Current status of the Botryosphaeriaceae in Australia. Australas. Plant Pathol. 48:35–44. doi:10.1007/s13313-018-0577-5

Central Weather Bureau. 2018. Climatological data annual report. Central Weather Bureau, Taipei, Taiwan. https://www.cwb.gov.tw/Data/service/notice/download/publish_20200421152340.pdf (visit on 04/29/2021)

Correia, K. C., M. A. Silva, M. A. de Morais, Jr., J. Armengol, A. J. L. Phillips, M. P. S. Câmara, and S. J. Michereff. 2016. Phylogeny, distribution and pathogenicity of *Lasiodiplodia* species associated with dieback of table grape in the main Brazilian exporting region. Plant Pathol. 65:92–103. doi:10.1111/ppa.12388

Dann, E. K., R. C. Ploetz, L. M. Coates, and K. G. Pegg. 2013. Foliar, fruit and soilborne diseases. p.380–422. *in*: The Avocado: Botany, Production and Uses. 2nd

ed. (Schaffer, B., B. N. Wolstenholme, and A. W. Whiley, eds.) CABI. Wallingford, UK. 560 pp.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: More models, new heuristics and parallel computing. Nat. Methods 9:722. doi:10.1038/nmeth.2109

Eskalen, A., B. Faber, and M. Bianchi. 2013. Spore trapping and pathogenicity of fungi in the Botryosphaeriaceae and Diaporthaceae associated with avocado branch canker in California. Plant Dis. 97:329–332. doi:10.1094/PDIS-03-12-0260-RE

Ghosh, S. P. 2000. Avocado production in India. p.24–30. *in*: Avocado Production in Asia and the Pacific. (Papademetriou, M. K., ed.) Food and Agriculture Organization of the United Nations and Regional Office for Asia and the Pacific. Bangkok, Thailand. 72 pp.

Guarnaccia, V., A. Vitale, G. Cirvilleri, D. Aiello, A. Susca, F. Epifani, G. Perrone, and G. Polizzi. 2016. Characterisation and pathogenicity of fungal species associated with branch cankers and stem-end rot of avocado in Italy. Eur. J. Plant Pathol. 146:963–976. doi:10.1007/s10658-016-0973-z

Guarnaccia, V., M. Sandoval-Denis, D. Aiello, G. Polizzi, and P. W. Crous. 2018. *Neocosmospora perseae* sp. nov., causing trunk cankers on avocado in Italy. Fungal Syst. Evol. 1:131–140. doi:10.3114/fuse.2018.01.06

Jacobs, K., D. R. Bergdahl, M. J. Wingfield, S. Halik, K. A. Seifert, D. E. Bright, and B. D. Wingfield. 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycol. Res. 108:411–418. doi:10.1017/S0953756204009748

Kee, Y. J., N. N. Suhaimi, L. Zakaria, and M. H. Mohd. 2017. Characterisation of *Neoscytalidium dimidiatum* causing leaf blight on *Sansevieria trifasciata* in Malaysia. Australas. Plant Dis. Notes 12:60. doi:10.1007/s13314-017-0284-z

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. doi:10.1093/bioinformatics/btm404

Marques, M. W., N. B. Lima, M. A. de Morais, Jr., M. A. G. Barbosa, B. O. Souza, S. J. Michereff, A. J. L. Phillips, and M. P. S. Câmara. 2013. Species of *Lasiodiplodia* associated with mango in Brazil. Fungal Divers. 61:181–193. doi:10.1007/s13225-013-0231-z

McDonald, V. and A. Eskalen. 2011. Botryosphaeriaceae species associated with avocado branch cankers in California. Plant Dis. 95:1465–1473. doi:10.1094/PDIS-02-11-0136

Netto, M. S. B., I. P. Assunção, G. S. A. Lima, M. W. Marques, W. G. Lima, J. H. A. Monteiro, V. de Queiroz Balbino, S. J. Michereff, A. J. L. Phillips, and M. P. S. Câmara. 2014. Species of *Lasiodiplodia* associated with papaya stem-end rot in Brazil. Fungal Divers. 67:127–141. doi:10.1007/s13225-014-0279-4

Ni, H. F., M. F. Chuang, S. L. Hsu, S. Y. Lai, and H. R. Yang. 2011. Survey of *Botryosphaeria* spp., causal agents of postharvest disease of avocado, in Taiwan. J. Taiwan Agric. Res. 60:157–166. (in Chinese with English abstract)

Phillips, A. J. L., A. Alves, J. Abdollahzadeh, B. Slippers, M. J. Wingfield, J. Z. Groenewald, and P. W. Crous. 2013. The *Botryosphaeriaceae*: Genera and species known from culture. Stud. Mycol. 76:51–167. doi:10.3114/sim0021

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61:539–542. doi:10.1093/sysbio/sys029

Rosado, A. W. C., A. R. Machado, F. D. C. O. Freire, and O. L. Pereira. 2016. Phylogeny, identification, and pathogenicity of *Lasiodiplodia* associated with postharvest stem-end rot of coconut in Brazil. Plant Dis. 100:561–568. doi:10.1094/PDIS-03-15-0242-RE

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. doi:10.1016/j.fbr.2007.06.002

Trakunyingcharoen, T., R. Cheewangkoon, and C. To-Anun. 2015. Phylogenetic study of the *Botryosphaeriaceae* species associated with avocado and pará rubber in Thailand. Chiang Mai J. Sci. 42:104–116.

Twizeyimana, M., H. Förster, V. McDonald, D. H. Wang, J. E. Adaskaveg, and A. Eskalen. 2013. Identification and pathogenicity of fungal pathogens associated with stem-end rot of avocado in California. Plant Dis. 97:1580–1584. doi:10.1094/PDIS-03-13-0230-RE

Vaidya, G., D. J. Lohman, and R. Meier. 2011. SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27:171–180. doi:10.1111/j.1096-0031.2010.00329.x

Valencia, A. L., P. M. Gil, B. A. Latorre, and I. M. Rosales. 2019. Characterization and pathogenicity of Botryosphaeriaceae species obtained from avocado trees with branch canker and dieback and from avocado fruit with stem end rot in Chile. Plant Dis.

103:996–1005. doi:10.1094/PDIS-07-18-1131-RE

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p.315–322. *in*: PCR Protocols: A Guide to Methods and Applications. (Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White, eds.) Academic Press. Cambridge, MA. 482 pp.

Zea-Bonilla, T., M. A. González-Sánchez, P. M. Martín-Sánchez, and R. M. Pérez-Jiménez. 2007. Avocado dieback caused by *Neofusicoccum parvum* in the Andalucia region, Spain. Plant Dis. 91:1052. doi:10.1094/PDIS-91-8-1052B

Lasiodiplodia theobromae 及 *Lasiodiplodia pseudotheobromae* 在台灣引起之酪梨枝條潰瘍病

梁鈺平[1] 吳昭蓉[1] 蔡惠文[2] 倪蕙芳[3,*]

摘要

梁鈺平、吳昭蓉、蔡惠文、倪蕙芳。2021。*Lasiodiplodia theobromae* 及 *Lasiodiplodia pseudotheobromae* 在台灣引起之酪梨枝條潰瘍病。台灣農業研究 70(2):81–97。

酪梨枝條潰瘍病於國外許多酪梨栽培國家均曾報導，主要由多種葡萄座腔菌科 (Botryosphaeriaceae) 之真菌感染引起。本病亦廣泛發生於台灣主要酪梨栽培地區，然病原菌至今尚不明確。本研究自台灣各地共 7 個酪梨園具潰瘍病病徵之枝條組織進行病原菌分離，共收集到 13 個菌株，透過型態鑑定及核糖體內轉錄區間 (internal transcribed spacer; ITS) 和轉譯延長因子 1-α (translation elongation factor 1-α gene; TEF-1α) 之序列進行類緣分析，結果顯示此些菌株均屬於 *Lasiodiplodia theobromae* 或 *Lasiodiplodia pseudotheobromae*。此兩菌株之菌絲最適生長溫度均為約 30℃；對酪梨枝條及果實均具病原性，可於枝條接種處造成白色粉末分泌及內部組織褐化，於果實上亦會造成黑色軟腐病斑。本研究為首篇描述台灣酪梨枝條潰瘍病及其相關病原菌之報告，研究結果顯示台灣之病原菌種類與大部分國外酪梨栽培地區造成酪梨枝條潰瘍病之主要病原菌不同。本研究將可提供未來進行酪梨枝條潰瘍病之流行病學，以及建立其有效之病害管理策略研究時之重要參考資訊。

關鍵詞：酪梨、枝條潰瘍病、*Lasiodiplodia theobromae*、*Lasiodiplodia pseudotheobromae*、台灣。

投稿日期：2020 年 11 月 19 日；接受日期：2020 年 12 月 28 日。
* 通訊作者：hfni@dns.caes.gov.tw
[1] 農委會農業試驗所嘉義農業試驗分所植物保護系助理研究員。台灣 嘉義市。
[2] 農委會農業試驗所嘉義農業試驗分所園藝系聘用助理研究員。台灣 嘉義市。
[3] 農委會農業試驗所嘉義農業試驗分所植物保護系副研究員兼系主任。台灣 嘉義市。