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Ecology and Epidemiology

Prevalence, Identity, Pathogenicity, and Infection Dynamics of Botryosphaeriaceae Causing Avocado Branch Canker in California

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Abstract

Botryosphaeria branch canker and dieback of avocado (*Persea americana* Mill.) has expanded in avocado-growing areas in recent years. Twenty-one avocado groves in the major producing regions of California were surveyed in 2018 and 2019. Monthly inoculations of wounded, green, and lignified branches of 'Hass' and 'Lamb Hass' were conducted. Botryosphaeriaceae were the predominant fungi recovered from cankered tissues collected across the surveyed traditional and high-density orchards and caused symptoms on all six sampled cultivars. These fungi were also recovered in asymptomatic twigs and other organs and thus exist as a potential reservoir for future infections. Molecular analyses of 173 isolates showed that *Neofusicoccum luteum* had the greatest incidences across sites and cultivars, with 83 and 29% recovered from Hass and Lamb Hass, respectively. Pathogenicity tests on excised (Hass, GEM, and Hass mutants) and attached shoots from potted (Hass) and mature avocado trees (Hass and Lamb Hass) showed that all species were pathogenic on wounded, green, and mature branches of the specified cultivars. Monthly inoculations of wounded, green, and lignified branches of Hass and Lamb Hass showed that both stem types were susceptible throughout the inoculation periods, regardless of the avocado phenological stage. In temperature-dependent growth and infection studies, growth of three points could vary during the growing season. Botryosphaeriaceae grown was higher between 20 and 30°C, but only *Lasiodiplodia theobromae* significantly grew and caused external lesions at 35°C. *Lasiodiplodia theobromae* also grew more on perseitol-amended media, all indicating its adaptation to warmer temperatures and capacity in metabolizing the avocado-produced sugar. Overall, this study extended our knowledge of the prevalence, identity, and pathogenicity of Botryosphaeriaceae on avocado cultivars, which will be useful to tailor management strategies.

Keywords: abiotic factors, avocado orchards, Botryosphaeriaceae, cultivar susceptibility, dieback, inoculum sources, pathogenicity, perseitol

Commercial production of avocado (*Persea americana* Mill.) in the United States ranked eighth in 2018 global production, with 100,000 tons (Altendorf 2019; FAOSTAT 2019). The majority of U.S. avocados are grown in California, with about 92% of U.S. avocados produced in California during the 2018 to 2019 season (USDA ERS 2020). In the 2019 to 2020 growing season, the California avocado industry occupied over 46,000 acres of producing orchards, planted mainly in San Diego, Riverside, Ventura, and San Luis Obispo counties, with an annual value of over $411 million (California Avocado Commission 2020).

Avocado production is decreasing worldwide due to diseases (Pérez-Jiménez 2008), including canker diseases. In most avocado groves, avocado branch canker and dieback, also known as avocado branch canker (ABC), is increasingly being recognized as a major issue for avocado production worldwide (Auger et al. 2013; Carrillo et al. 2016; McDonald and Eskalen 2011; Valencia et al. 2019; Zea-Bonilla et al. 2007). Over the past several years, California avocado growers, private consultants, and extension specialists have all noted that ABC is increasingly common in the state's avocado-growing areas. A statewide survey of mature avocado orchards, conducted more than 10 years ago, has shown a widespread occurrence of ABC in avocado-producing counties of California (McDonald and Eskalen 2011). These increased disease incidences have been related to adverse conditions caused by prolonged drought, as seen in California in recent years. A taxonomic study of the pathogens using molecular and phylogenetic techniques further revealed that a diversity of fungal species within the Botryosphaeriaceae family are the main causal agents of this disease (McDonald and Eskalen 2011). These pathogens can survive as parasites or saprophytes, but many are latent pathogens that may live undetected in asymptomatic tissue of woody shrubs and trees until symptoms are expressed when the host is weakened following exposure to stressful conditions (Amponsah et al. 2014; McDonald and Eskalen 2011; Slippers and Wingfield 2007; Twizeyimana et al. 2013; Van Niekerk et al. 2011). These fungi overwinter on the surface of diseased wood under the bark, as pycnidia (small, dark, "pimple-like" asexual structures), which release water-splashed conidia (Ahimera et al. 2004; Eskalen et al. 2013). Rarely, they also form flask-shaped sexual fruiting bodies (pseudothecia) on the outside of cankers, producing sexual fungal spores (ascospores) disseminated by wind and rain splash (Ahimera et al. 2004; Eskalen et al. 2013). Botryosphaeriaceae can attack a wide range of woody plants through lenticels, but they mainly infect the host via fresh pruning wounds, invading the xylem vessels. Symptomatic branch cankers exhibit necrotic, friable bark, red-brown cankers, and branch dieback associated with characteristic whitish exudate of perseitol, whereas internally, the wood becomes reddish brown (Dann et al. 2013; McDonald and Eskalen 2011). Infections by Botryosphaeriaceae fungi can ultimately produce extensive damage, cause tissue death, and reduce the productivity of the orchard.

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Although the abovementioned studies on Botryosphaeriaceae species attacking 'Hass' avocado in California have shed light on their identity, diversity, and pathogenicity (McDonald and Eskalen 2011; Twizeyimana et al. 2013), updated and improved knowledge regarding their prevalence, pathogenic life, and disease cycles in California avocado groves is still needed to develop and apply appropriate ABC control measures. Hence, regular surveys of avocado orchards throughout the main avocado-producing regions are needed to learn more about the occurrence and distribution of the causal Botryosphaeriaceae species and their impact on avocado cultivars. Development of disease-resistant avocado varieties could be a valuable and more proactive choice in managing ABC. Three ecological races or botanical varieties of avocado adapted to different climate conditions have traditionally been recognized: Mexican [*P. americana* var. *drymifolia* (Schlecht. & Cham.) Blake], Guatemalan (*P. americana* var. *guatemalensis* L. Wms.), and West Indian (*P. americana* var. *americana* Mill.) (Bergh and Lahav 1996). The most important and abundant avocado cultivars grown in Californian orchards and worldwide, such as Hass, are Guatemalan × Mexican hybrids with different degrees of hybridization (Chanderbali et al. 2013; Newett et al. 2002). Previous pathogenicity studies on Hass avocado stems have shown its susceptibility to Botryosphaeriaceae (Guarnaccia et al. 2016, 2020; McDonald and Eskalen 2011; Twizeyimana et al. 2013; Valencia et al. 2019). However, information on their pathogenic and disease life cycles, including the occurrence of latent infections and virulence of the pathogens on newly introduced cultivars such as 'GEM' and 'Lamb Hass', or other unreleased related varieties, is lacking. Although pruning wounds are known to be important entry sites for these pathogens, little is known about the effect of wounding on infection and colonization of avocado shoots at different times during the avocado-growing season. In general, these pathogens infect lignified tissues and, less frequently, fruit, leaves, and flowers (Teviotdale et al. 2002; Trapero et al. 2011), but field studies with regard to infection of avocado branches of different phenological stages in relation to prevailing weather conditions, and contributing factors are still lacking.

Cankers on avocado may exude the whitish-beige powder perseitol, which, with *D*-mannoheptulose, is a heptose carbohydrate synthesized by avocado. These sugars are found in all parts of the avocado plant, but at different concentrations, depending on the tissue type and phenological stage of the plant (Liu et al. 1999). In agreement with these findings, our field observations of artificially infected shoots suggest that perseitol amounts at wound-inoculation points may vary during the growing season (H. F. Avenot, *personal observation*). Virulence of Botryosphaeriaceae in relation to the seasonal concentration fluxes of reserve starch in the woody tissues of the avocado tree is unknown. In some studies, carbohydrate levels and translocations in host tissues were shown to be affected canker pathogen infections (Li et al. 2019), but no study has investigated how variations in perseitol concentration may affect or stimulate the growth of Botryosphaeriaceae during infection of avocado.

The overall aim of the present study was to learn more about the prevalence, diversity, and pathogenic lifecycle of Botryosphaeriaceae species in mature avocado orchards in the main producing areas of California. The specific objectives were to (i) determine the prevalence and availability of primary and secondary sources of inocula of Botryosphaeriaceae in infected avocado tissues and debris from various cultivars; (ii) identify the most common species involved and assess their virulence on detached avocado organs and whole plants; (iii) compare the susceptibility of unreleased avocado cultivars with that of the most virulent species; (iv) study the effect of wounding on Botryosphaeriaceae seasonal infection and colonization of green and lignified avocado branches corresponding to two different phenological stages; and (v) assess the in vitro effects of a range of temperatures and different concentrations of perseitol on pathogen mycelial growth.

Materials and Methods

Field sampling, fungal isolation from infected and symptomless avocado tissues/debris, and morphological identification

Field surveys were conducted in the fall of 2018 and spring of 2019 in 22 commercial and experimental avocado orchards located throughout the main avocado-producing counties of California, namely San Diego Co. (seven orchards), Ventura Co. (five orchards), Riverside Co. (three orchards), San Luis Obispo Co. (four orchards), and Tulare Co. (one orchard) (Table 1). The main avocado variety planted in most counties is Hass. Most ($n = 21$) sampled orchards consisted of traditionally planted groves at a 20 × 20-foot spacing (109 trees per acre), whereas only one, located in Ventura, was a high-density orchard, in which closer spacings (300 to 500 trees per acre) are used (Rolshausen et al. 2016). Symptomatic and/or healthy trees were randomly selected from each orchard. Cankered and/or thinned symptomless tissues were sampled from 10 to 50 branches showing typical branch canker and dieback symptoms. Two experimental orchards located at the Lindcove Research and Extension Center (LREC) of the University of California, in Exeter, California (Tulare Co.), and the Pine Tree Ranch (PTR) (California Pomona University) in Santa Paula (Ventura Co.) were also surveyed in the fall of 2018 and/or spring of 2019. The well-known commercial variety Hass is grown at the PTR, along with Lamb Has' (Guatemalan × Mexican), and Hass, GEM (Guatemalan) (Martin and Bergh, US Patent US14), 'Carmen' (Mexican), and other unreleased Hass-related varieties are grown at LREC. The samples were transported back to the laboratory in an ice chest and stored at 5°C until processed for isolations of putative pathogens. Wood samples included portions of blighted twigs or shoots and cankered branches or trunks with or without sunburn damages. In addition to cankered twigs and branches, samples of green twigs, other infected or asymptomatic avocado organs (leaves, fruits, flowers, peduncles, petioles, pedicels), and litter from the ground were also collected periodically from randomly chosen Hass trees to check for pathogen presence, occurrence of latent infections, or fruiting structures. Detached, symptomless organs were cut individually into small pieces (4 × 5 mm) (leaves, petioles, fruits, and peduncles) or into approximately 4-cm-long segments (twigs). Diseased and asymptomatic tissues were surface disinfected with household bleach (Mfd. for Clorox Professional Product Company, Oakland, CA) at 10% (vol/vol) in sterile water for 3 min, rinsed with sterile distilled water, and allowed to air dry in a laminar flow hood. Fungal isolations were done by plating either small pieces of asymptomatic organs or both necrotic and healthy tissue parts from diseased organs in Petri dishes containing 2% potato dextrose agar (PDA) (Microtech Scientific, Orange, CA; 10 g of PDA, 500 ml of water) acidified (APDA) with lactic acid (2.5 ml of 25% [vol/vol] per liter of medium) to minimize bacterial growth (Chen et al. 2014). Petri dishes were incubated at 25°C for 3 to 7 days until fungal colonies were large enough to be examined. Pure cultures were obtained by transferring single hyphal tips from the colonies to fresh APDA and incubated at 25°C for 7 to 10 days. Fungal isolates were identified to genus based on typical colony morphology (color, growth pattern, and rate) and conidial characteristics (Chen et al. 2014; Crous et al. 2006; Phoulivong et al. 2010; Valencia et al. 2019). They were maintained as mycelial plugs in sterile water at 4°C in our fungal collection at the University of California, Kearney Agricultural Research and Extension Center (UC KARE), Parlier, California. Presence of Botryosphaeriaceae fruiting structures, namely pycnidia and pseudothecia, in naturally occurring cankers on live stems or pruning debris was monitored sporadically during each sampling period. When found on affected tissue, fruiting structure was removed using a sterile needle under a dissecting microscope and mounted for viewing of the conidia or asci/ascospores in a compound microscope.

Molecular identification of Botryosphaeriaceae recovered from various avocado tissues

To identify isolates of Botryosphaeriaceae and other genera (*Colletotrichum*, *Phomopsis*, and *Fusarium*) to species and assess their distribution on avocado organs and in orchards, one to two isolates were randomly selected from each of the sampled orchards. Some Botryosphaeriaceae isolates isolated from infected samples collected during an initial survey conducted in 2017 were also included. Genomic DNA (gDNA) was extracted from an axenic culture of each isolate by using the FastDNA Kit (MP Biomedicals, Burlingame, CA) in conjunction with the FastPrep-24 Instrument (MP Biomedicals, Irvine, CA) according to the manufacturer's instructions. Purified gDNA samples were quantified with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE), diluted to 5 ng/µl for PCR, and stored at −20°C until use. Partial gene regions of the translation elongation factor 1-alpha (TEF-1a) for Botryosphaeriaceae, *Phomopsis*, and *Fusarium* and β-tubulin (TUB2) for *Colletotrichum* isolates were amplified using the primer sets EF1-728F/EF1-986R (Carbone et al. 1999) and BT2-A/BT2-B (Glass and Donaldson 1995), respectively. The polymerase chain reactions (PCRs) were performed in a total volume of 20 µl in AccuPower PCR premix tubes (Bioneer, Alameda, CA). Each PCR mixture contained 10 ng of gDNA, 0.5 µl of 10 µmol of each primer, and 17 µl of DNase-free water. PCR amplifications of TEF and TUB2 products were conducted in a T100 Thermal Cycler (Bio-Rad, Hercules, CA) and started with an initial denaturation cycle of 3 min at 95°C, followed by 35 cycles of 30 s at 95°C, 45 s at 55°C for the (TEF) and 52°C for the (TUB2), then 1 min at 72°C, and a final elongation step of 6 min at 72°C. PCR products were separated using electrophoresis and purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) following the manufacturer's instructions. Purified products were then sequenced in both directions using the primers mentioned above at the Division of Biological Sciences sequencing facility of the University of California, Davis. The nucleotide sequences were edited using BioEdit Sequence Alignment. To identify isolates of Botryosphaeriaceae and other detected fungi to species, the obtained TEF-1a and TUB2 consensus sequences were subjected to BLASTn search using the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) nucleotide database. Percentages of fungal species recovered were then calculated. Selected sequences of the studied Botryosphaeriaceae isolates were then compared and aligned with those of Botryosphaeriaceae species published in NCBI, using the mega 5.05 software (http://www.megasoftware.net/). Phylogenetic analysis of the TEF sequences was conducted using the neighbor-joining method. The evolutionary distances were computed using the Kimura 2-parameter method, and all positions containing gaps and missing data were eliminated.

Pathogenicity of Botryosphaeriaceae isolates on avocados

Pathogenicity assays on detached avocado organs and whole plants were performed to determine the ability of the identified fungal species to produce cankers.

Plant material. Pathogenicity in laboratory conditions was assessed on 1-year-old detached, green shoots collected from healthy-looking avocado trees located at LREC. Shoots of Hass and GEM varieties used in virulence tests were collected in April 2019. Shoots of various unreleased Hass-related mutants (codes 1 to 7) used in cultivar susceptibility experiments were first collected in April 2020 and then in April 2021. The effect of temperature on fungal colonization was examined performed on asymptomatic Hass twigs collected from avocado trees at the PTR. Leaves were removed

TABLE 1. Incidence of Botryosphaeriaceae and other fungi in cankered avocado branches collected in 2018 and 2019 from avocado orchards in several producing counties of California

County	City	Orchards	Date of sampling[z]	Cultivar	Branch cankers	Pathogen incidence (%) in cankered branch samples				
						Botryosphaeriaceae	*Colletotrichum*	*Phomopsis*	*Alternaria*	*Fusarium*
Riverside	Riverside	RC1	Nov 2018	'Hass'	192	48.9	16.7	0	34.4	11.5
		RC2			101	41.6	1	10.9	29.7	21.8
		RC3			50	26	4	0	56	12
Ventura	Pomona	PTR	Nov 2018	'Hass'	263	62.4	17.9	0	7.2	8.7
			Apr 2019	'Lamb Hass'	50	50	4	0	54	0
	Fillmore	G3	Nov 2018	'Hass'	104	83.6	8.6	0	0	4.8
	Santa Paula	LIM high-density			162	21.0	29.6	9.2	4.3	26.5
		LIM-O			32	53.1	43.7	0	0	0
		DA1			180	50.0	25.6	0	12.2	0.6
San Diego	Bonsall	West-L	Nov 2018	'Hass'	117	79.5	11.1	0	0.8	7.7
	Valley Center	ZRT			42	57.1	16.7	0	0	9.5
		Mesa-C			63	28.6	20.6	3.2	14.3	15.9
	Pauma Valley	Starbeam			51	19.6	5.9	2	29.4	39.2
	Fallbrook	NIG			32	6.2	9.4	0	15.6	68.7
	Bonsall	West-L		'Lamb Hass'	94	67	26.6	0	9.6	14.9
	Pauma Valley	Starbeam			53	75.5	5.7	0	7.6	15.1
San Luis Obispo	Morro Bay	GR1	Nov 2018	'Hass'	38	90	14	0	0	0
	San Luis Obispo	GR2			223	81.6	9.4	0	0.9	0.4
	Morro Bay	GR1	Apr 2019		161	67.1	23.6	9.9	5.6	2.5
		GR3			100	85	5	1	2	6
	Cayucos	GR4			50	24	24	14	16	4
	San Luis Obispo	GR2			163	81.6	7.4	14.1	4.9	9.2
Tulare	Exeter	Lindcove Station	Nov 2018	'Hass'	70	1.4	0	0	35.7	4.3
				'Gem'	10	10	0	0	70	0
				'Carmen'	30	3.3	6.7	0	13.3	0
			Apr 2019	'Hass'	40	27.5	0	0	20	0
				'Gem'	57	21	1.7	0	64.9	21
				cv. code-1	10	10	0	20	40	0
				cv. code-5	20	35	0	20	35	0
				'Carmen'	10	0	0	30	10	0
Total/average incidence					2,565	43.6	13.3	4.5	19.8	10.1

[z] Samples collected in November 2018 and April 2019 included symptomatic and asymptomatic twigs or branches (with or without sunburn damage).

from the shoots, and branch fragments of approximately 25 cm in length were excised using pruning shears. Both fragment ends were dipped into paraffin wax to prevent desiccation. For pathogenicity on detached avocado fruits, asymptomatic mature avocado Hass and Lamb Hass fruits were randomly collected in October 2020 from the PTR avocado experimental orchard. All plant samples were maintained in a cold room (set at 5°C) at UC KARE until they were processed in the laboratory.

For pathogenicity in the lathhouse, 2-year-old potted avocado trees (Hass grafted on 'Duck 7' rootstock) were obtained from Brokaw Nursery (Ventura, CA) and maintained at UC KARE before use.

Pathogenicity in field conditions was performed on branches from healthy-looking 7-year-old Hass and 7-year-old Lamb Hass trees located at the PTR orchard. The avocado site is irrigated via microsprinklers.

Fungal material. A total of 13 isolates, identified during the survey and consisting of six Botryosphaeriaceae, three *Colletotrichum*, and one *Phomopsis* species, were randomly selected and used in laboratory, lathhouse, and field inoculations, as specified in Table 2. An isolate of *C. fioriniae* (12D46) isolated from a pistachio orchard in California was also added in the lathhouse experiment.

Inoculation procedure. Prior to inoculation, detached organs (25-cm fragments and fruits) were surface-sterilized in 10% sodium hypochlorite solution for 3 min, rinsed twice with sterile distilled water, and air dried in a laminar flow hood over clean paper towels. Shoots were immediately sealed at both ends with Parafilm (Bemis Company, Neenah, WI) to reduce desiccation.

In the detached twig experiment, Hass and GEM shoots were inoculated with several Botryosphaeriaceae species, whereas in the cultivar susceptibility experiment, shoots from other avocado varieties, along with those of Hass and GEM were inoculated with one aggressive isolate (Lth-HA37) of *L. theobromae* (Table 2). Several isolate species were used for inoculating Hass and/or Lamb Hass shoots in the lathhouse and field experiments (Table 2). The detached inoculated twig segments were placed on sterile plastic racks in two humid chambers (plastic crispers, 31 × 23 × 10 cm) at room temperature and 100% relative humidity for 2 weeks. The experiments were set up as completely randomized (in virulence and cultivar susceptibility tests) designs with six replicate shoots per fungal isolate and per humid chamber, and the experiment was repeated twice. Twelve control twigs in two humid chambers (six shoots in each) were inoculated with sterile PDA plugs (8-mm diameter) to serve as controls. For each treatment, internal lesion length that developed in twigs was measured 2 weeks after inoculation.

Detached shoots, attached Hass shoots from potted trees, and attached shoots on Hass and Lamb Hass trees, in the laboratory, lathhouse, and field, respectively, were wounded (1 to 2 mm deep) using an 8-mm cork borer to remove the outer bark and expose the underlying cambium. Mycelial plugs (8-mm diameter) from the actively growing margin of the fungal cultures were placed mycelial side down in the wounded tissues.

Wounds on attached branches, in both lathhouse and field experiments, were covered with petroleum jelly and wrapped with Parafilm. Five wounded branches were used for each isolate in completely randomized designs for both lathhouse and field experiments, which were repeated twice. Ten branches were inoculated with sterile agar plugs to serve as controls. The length of the internal necrotic lesions on the inoculated branches was measured 2 months after inoculation.

The pathogenicity on detached Hass and Lamb Hass avocado fruits was tested using one virulent isolate of *L. theobromae* (Table 2). Twenty mature fruits per cultivar were collected from arbitrarily selected trees. Inoculations were made by creating a wound of approximately 6 mm wide × and 4 mm deep with a 6-mm-diameter cork borer on the center of each fruit. Immediately after wounding, 10 fruits from each cultivar were inoculated by inserting a 6-mm-diameter mycelial plug of actively growing colonies of isolate Lth-HA37 into each wound. Ten fruits were wounded and inoculated with sterile PDA plugs and used as a negative control. All fruits were incubated in humid chambers (100% relative humidity) for 10 days at room temperature. The experiment was repeated twice. The experimental design was a completely randomized design with 10 replicates of each treatment. The diameter of the necrotic lesion of each fruit was recorded 10 days after inoculation.

Influence of wounding at different times of the year on the infections of green and lignified avocado shoots by Botryosphaeriaceae

Field experiments were established between May 2019 and December 2020 at the PTR to assess the influence of wounding at different times of the year on the infections of green and lignified avocado shoots by Botryosphaeriaceae. Monthly, artificial inoculations were conducted on shoots of 7-year-old Hass and 7-year-old Lamb Hass avocado trees (both on 'Toro canyon' rootstock) by using the same mycelial plug inoculation method described above. The selected shoots (green and lignified) corresponded to different phenological stages of avocado twig development. Inoculations were carried out on wounded, green avocado branches from May through December 2019 and on wounded lignified shoots from January to December 2020. The two aggressive isolates of Botryosphaeriaceae (*L. theobromae*, Lth-HA37, and *N. nonquaesitum*, Nnq-HA1) isolated from avocado were used. Each isolate was inoculated on 10 green (1- to 2-year-old; ~1.5- to 2-cm diameter) and 10 lignified (~3- to 4.5-cm diameter) branches (five shoots per cultivar tree) and arranged in a completely randomized design. Ten additional branches (five per tree) of each cultivar were inoculated

TABLE 2. Characteristics of fungal isolates used (+) in various pathogenicity tests[z]

Species	Name	Origin			Detached	Lathhouse	Field
		City (county)	Host	Organ			
Botryosphaeria dothidea	Bdot-HA51	Santa Paula (Ventura)	Avocado	Twig	+	+	+
Lasiodiplodia theobromae	Lth-HA37	Santa Paula (Ventura)		Trunk	+	+	+
Neofusicoccum mediterraneum	Nmed-HA60	Pomona (Ventura)		Petiole	+	+	+
N. nonquaesitum	Nnq-HA1	San Luis Obispo (San Luis Obispo = SLO)		Twig	+	+	+
N. nonquaesitum	Nnq-HA27	San Luis Obispo (SLO)		Twig	+	−	−
N. luteum	Nl-HA46	Bonsall (San Diego)		Twig	+	+	+
N. australe	Na-HA19	San Luis Obispo (SLO)		Twig	+	+	+
N. australe	Na-HA26	San Luis Obispo (SLO)		Twig	+	−	−
B. dothidea	Bot-HA36	Bonsall (San Diego)		Twig	+	−	−
Colletotrichum siamense	Cs-HA65	Santa Paula (Ventura)		Leaf	+	+	−
C. fructicola	Cf-HA87	Santa Paula (Ventura)		Leaf	+	+	+
C. gloeosporioides	Cg-HA94	Santa Paula (Ventura)		Leaf	+	+	+
Phomopsis fukushii	Pf-HA5	San Luis Obispo (SLO)		Twig	+	−	−
C. fiorinae	12D46	California	Pistachio	Fruit	−	+	−

[z] + indicates that the isolate was used in the specified pathogenicity tests; − indicates that the isolate was not used in the specified pathogenicity tests.

with sterile 2% 8-mm PDA plugs and served as negative controls. Inoculated and control branches were removed from the orchards 2 (green shoots) and 4 months (lignified branches) after each inoculation time. The internal lesion length on avocado branches from each cultivar-isolate combination was then measured. Disease incidence was calculated by dividing the number of infected shoots by the total number of inoculated shoots. Observations of the avocado phenological stage(s) were made at each inoculation time to follow activity of infection in relation to avocado phenology.

Koch's postulates. To fulfill Koch's postulates, pathogenicity assays were conducted by inoculating 10 replicate wounded shoots per treatment/isolate organized in a completely randomized design. Shoots inoculated with sterile agar plugs served as negative controls. Shoots were inspected for the development of necrotic lesions, the diameter of which was measured 2 weeks postinoculation. To recover the inoculated fungal isolates, small pieces of tissue obtained from the margins of necrotic or vascular lesions of inoculated shoots and from uninoculated control shoots were placed on APDA and incubated at 25°C for 3 to 7 days. The reisolated fungi from symptomatic tissues were reidentified by cultural and morphological comparison with the original isolates.

Effects of perseitol and temperature on Botryosphaeriaceae mycelial growth and/or colonization of detached avocado twigs

To evaluate the effect of temperature on radial growth of Botryosphaeriaceae isolates, mycelial plugs (8 mm) were cut from active PDA cultures and transferred to the center of new PDA plates. A total of three isolates from three species of Botryosphaeriaceae, namely *L. theobromae* (Lth-HA37), *N. nonquaesitum* (Nnq-HA1), and *N. australe* (Na-HA38), were selected. These cultures were incubated at six different temperatures (10, 15, 20, 25, 30, and 35°C) in the dark for 3 days. The experiments were set up as a completely randomized design with three replicate plates per temperature and per each isolate, and the experiment was conducted twice. Colony diameters were recorded after 3 days in two orthogonal directions.

Similarly, the effect of various concentrations (0, 1, 10, 100, and 200 μg/ml) of perseitol (Combi-Blocks, San Diego, CA) on the radial growth of Botryosphaeriaceae and other fungal isolates was analyzed. Three replicate plates (containing 5 g of peptone and 7.5 g of agar in 500 ml) per each fungal isolate and concentration were used, and the experiment was conducted twice. Plates were incubated at 25°C for 6 days, and colony diameters were then recorded in two orthogonal directions.

To assess the effect of temperature on fungal colonization, Hass twigs were sterilized, wounded, and then inoculated as described above with mycelial plugs from actively growing cultures of *L. theobromae* and *B. dothidea.* Control twigs were inoculated with sterile agar plugs, and all wounds were covered with petroleum jelly and wrapped with Parafilm. The experiment design was a two-level completely randomized design with temperature and isolate as factors. Inoculated and control twigs were placed in 100% humidity crispers and incubated at 25 or 35°C for 14 days. Each temperature and isolate combination had 10 replicate twigs, and the experiment was repeated twice. After incubation, external lesion length was measured, and then the bark in each fragment was removed to record internal vascular necrosis. Koch's postulates were fulfilled as described above.

Statistical analyses

Internal and/or external lesion lengths in laboratory, lathhouse, and/or field tests, fungal radial growths, and lesion lengths in temperature and perseitol studies were recorded. To check the normality and homogeneity of variance, Shapiro-Wilks and Levene's tests were applied on the standardized residuals of the raw data.

The effects of cultivar and fungal isolates on internal lesion length data in detached (Hass and GEM) and attached shoots (Hass and Lamb Hass) were analyzed using a two-way analysis of variance (ANOVA) for a two-factor completely randomized design. Statistical significance of the main and interaction effects was determined by comparing the P value for the F test to the significance level ($\alpha = 0.05$).

The effects of cultivar on internal lesion length in the cultivar susceptibility experiment on detached shoots and of isolate on lesion length in the lathhouse experiment were compared using a one-way ANOVA. Treatment means were separated by the least significant difference (LSD) test ($P = 0.05$).

The effects of temperature, fungal species, and the interaction of temperature × fungal species on mycelial growth rate were evaluated using a two-way ANOVA. Treatment means were compared according to the LSD test at $P = 0.05$.

Pathogenic differences among the inoculation/sampling months, for each isolate of the two Botryosphaeriaceae species on green and lignified shoots, were determined by recording the internal lesion lengths and calculating disease incidences. A preliminary ANOVA showed that there were no significant differences in terms of lesion length for each fungal species in each sampling month for each shoot type, so lesion data from each species were pooled. Data from the PTR orchard studies were subjected to a two-way ANOVA to assess the effect of inoculation month and isolate/control treatments and the interactions of month × isolate on the extent of vascular discoloration on green and lignified shoots. Statistical significance of the main and interaction effects was determined by comparing the P value for the F test to the significance level ($\alpha = 0.05$).

All statistical analyses were performed using Statistix 10 (Analytical Software, Tallahassee, FL).

Results

Incidence of Botryosphaeriaceae in cankered avocado branches/twigs and detection of latent infections and fruiting structures on infected plants/debris

Twenty-one avocado orchards were surveyed in the main avocado-producing counties in 2018/2019. A total of 2,568 branches/twigs with various symptoms were sampled from these orchards. The observed symptoms ranged from light brown lesions to dark cankers or V-shaped necrotic lesions. Laboratory analyses of symptomatic branches/twigs from 20 out of the 24 sampled orchards showed that Botryosphaeriaceae were the fungi predominantly isolated and associated with the necrotic or cankered branches and twigs of Hass and Lamb Hass (Table 1). Plating of the 2,568 wood pieces from all cultivars showed that the pathogen incidence ranged from 0 to 90% (mean = 43.6%), 0 to 43.7% (mean = 13.3%), and 0 to 30% (mean = 4.5%), for Botryosphaeriaceae, *Colletotrichum* spp., and *Diaporthe* (*Phomopsis*) spp., respectively (Table 1). The highest percentages of isolation of Botryosphaeriaceae were observed from commercial orchards of both Hass (90%) and Lamb Hass (75.5%) varieties. Pathogen incidence, following plating of Lamb Hass wood pieces (n = 197), ranged from 50 to 75.5% (mean = 64.2%), 4 to 26.6% (mean = 12.1%), and 7.6 to 54% (mean = 23.7%), respectively, for Botryosphaeriaceae, *Colletotrichum* spp., and *Alternaria* spp. (Table 1). Among 162 twig pieces collected in a high-density Hass avocado orchard (LIM-Y) in Santa Paula (Ventura Co.), Botryosphaeriaceae, *Colletotrichum*, and *Phomopsis* fungi were recovered at 21, 29.6, and 9.2%, respectively. Other recovered fungi included *Alternaria* and *Fusarium* spp.

Among the 247 tissue pieces from symptomatic twigs/branches of Hass, GEM, Carmen, and other unreleased cultivars (codes 4 and 7), collected in 2018/spring 2019 in Tulare County, pathogen incidence ranged from 0 to 35% (mean = 13.5%), 0 to 6.7% (mean = 1.05%), and 0 to 30% (mean = 8.8%), for Botryosphaeriaceae, *Colletotrichum* spp., and *Phomopsis* spp., respectively (Table 1).

Laboratory analyses of asymptomatic avocado shoots, petioles, fruits, and peduncles revealed that Botryosphaeriaceae could be isolated from apparently healthy, symptomless avocado tissues collected periodically starting in August of 2019, thus revealing the

presence of latent infections. Microscopic analysis of some of the collected samples, including soil debris and dead branches, showed the presence of both the pycnidia and the pseudothecia in avocado tissues (Table 4). Isolation and subsequent cultural observation of the growing fungi confirmed them as Botryosphaeriaceae. Thus, both the water-splashed and the airborne inocula are present in avocado orchards.

Incidence of Botryosphaeriaceae in infected avocado leaves

Laboratory analyses of symptomatic leaves revealed that *Alternaria* and *Colletotrichum* spp. were the pathogens predominantly isolated and associated with the various symptoms described above. Of 1,213 infected leaf samples collected in the orchards in all counties, 52.5, 28.4, and 13.5% yielded species of *Alternaria, Colletotrichum*, and Botryosphaeriaceae, respectively (Table 3). Of 61 infected leaf samples collected in the two Lamb Hass orchards in San Diego County, 61.2, 45.2, and 11.3% on average yielded species of *Alternaria*, *Colletotrichum*, and Botryosphaeriaceae, respectively. Plating of pieces of symptomatic leaves ($n = 50$) of the GEM cultivar, collected in the spring of 2019 in Tulare, yielded 10, 10, and 76% of Botryosphaeriaceae, *Colletotrichum*, and *Alternaria* spp., respectively (Table 3).

Incidence of Botryosphaeriaceae in other infected avocado tissues

Laboratory analyses of symptomatic avocado organs (petioles, fruits, pedicels, and peduncles) revealed that they were infected by Botryosphaeriaceae fungi across all counties and cultivars at low, moderate, and high proportions (Table 4). Mean percentages of recovery Botryosphaeriaceae from infected petioles, peduncles, and fruits were 18, 23.7, and 21, respectively (Table 4). Up to 88% of infected fruit from West-L from San Diego were infected with Botryosphaeriaceae.

Molecular identification and prevalence of Botryosphaeriaceae in avocado orchards

Molecular analyses of partial sequences of the translation elongation factor 1alpha gene (TEF-1α) regions for a total of 173 isolates recovered from the sampled orchards revealed about 14 known species of Botryosphaeriaceae and several undetermined species (Table 5). Species distributed throughout the sampled

TABLE 3. Incidence (%) of Botryosphaeriaceae species in symptomatic avocado leaves collected in 2018 and 2019 from several avocado orchards in several producing counties of California

				Pathogen incidence (%) in infected leaf samples				
Orchards	Year	Cultivar	Infected leaves	Botryosphaeriaceae	*Colletotrichum*	*Phomopsis*	*Alternaria*	*Fusarium*
RC1	2018	'Hass'	53	15.1	13.2	1.9	71.7	7.6
RC2			23	0	4.3	0	47.8	4.3
RC3			53	1.9	17.0	0	96.2	0
PTR	2018	'Hass'	20	0	0	0	80	0
	2019	'Lamb Hass'	150	2	1.3	10.7	72	6
G3	2018	'Hass'	30	20	23.3	3.3	56.7	0
LIM High density			81	0	18.5	0	83.9	2.5
LIM-O			11	27.3	63.6	9.1	0	0
DA1			120	15.0	31.7	0	50.8	0.8
West-L	2018	'Hass'	42	40.5	35.7	0	14.3	7.1
ZRT			10	0	0	0	100	0
Starbeam			15	0	0	0	46.7	53.3
NIG			41	7.3	68.3	0	22.0	2.4
West-L		'Lamb Hass'	31	22.6	90.3	0	29.0	0
Starbeam			30	0	0	0	93.3	3.3
GR1	2018	'Hass'	52	42.8	66.2	0	0	0
GR2			10	0	0	0	100	0
GR1	2019	'Hass'	101	18.8	73.3	1	10.9	5.9
GR3			50	46	12	4	22	0
GR4			80	6.2	76.2	8.7	52.5	1.2
GR2			160	21.9	25.6	4.4	28.1	0
Lindcove station	2019	'Gem'	50	10	4	10	76	0
Total/average incidence			1,213	13.5	28.4	2.4	52.5	4.3

TABLE 4. Incidence of Botryosphaeriaceae in other symptomatic avocado organs and debris collected in 2018 and 2019 from orchards in several producing counties of California

			Botryosphaeriaceae incidence (%) in avocado samples						
Orchard	Year	Cultivar	Infected petioles	Infected peduncles	Infected fruits	Fruit mummies	Infected pedicels	Soil debris	Infected inflorescences
RC1	2018	'Hass'	25	19.2	14.8	5	–	–	–
RC2			0	9.5	0	10	–	–	–
RC3			0	0	0	30	–	–	–
PTR	2018	'Hass'	–	10	–	–	–	–	–
DA1			39.6	3.5	–	–	–	–	–
PTR	2019	'Lamb Hass'	3.5	–	–	10	–	–	5
West-L	2018	'Hass'	–	–	88	–	–	–	–
NIG			3.3	35.7	–	–	–	–	–
Starbeam		'Lamb Hass'	1	–	2.4	1	–	–	–
GR1	2018	'Hass'	–	–	–	–	100	–	–
GR2			–	–	–	–	–	8.3	–
GR1	2019	'Hass'	20	56.7	–	12.5	–	–	24.4
GR3			16.7	55	–	–	–	–	30
GR2			34.5	–	–	–	–	100	1.7
GR4			54	–	–	0	0	–	5

counties included *Neofusicoccum luteum*, *N. nonquaesitum*, *N. australe*, *Botryosphaeria dothidea, N. mediterraneum*, *Lasiodiplodia theobromae*, and *N. parvum* (Table 5). Among all the 173 collected isolates, 124 (72%) were isolated from wood tissues, whereas 49 (28%) were recovered from other avocado organs, including leaf, fruit, petiole, and inflorescence tissues (Table 5). Among 11 Botryosphaeriaceae isolates recovered from Lamb Hass wood tissues, 91% were *N. luteum* (Table 5). Among 161 isolates isolated from Hass tissues (wood tissues and other avocado organs), *N. luteum* was the species with the greatest incidence (29%) across the sampled orchards, followed by *N. australe* (19%), *N. parvum* (15%), *N. nonquaesitum* (9%), *L. theobromae* (6%), *N. mediterraneum*, and *B. dothidea* (5.6%) (Table 5).

Phylogenetic analysis using sequences of the elongation factor 1-alpha (EF-1α) partial region from 47 isolates confirmed the 14 species of Botryosphaeriaceae as identified by BLASTn query results: *Neofusicoccum luteum* (12 isolates), *N. nonquaesitum* (11 isolates), *N. australe* (seven isolates), *N. cryptoaustrale* (four isolates), *Botryosphaeria dothidea* (four isolates), *N. mediterraneum* (three isolates), *Lasiodiplodia theobromae* (two isolates), *N. parvum* (one isolate), *Diplodia pseudoseriata* (one isolate), *D. corticola* (one isolate), and *D. africana* (one isolate). Two methods of phylogenetic reconstruction were compared and showed the same topology, but only the neighbor-joining tree is shown (Supplementary Fig. S1). The trees confirmed the identification by BLASTn, except for two species: first, the *N. cryptoaustrale* cluster, where three isolates were previously identified as *N. australe* and one as *N. luteum*, and the isolate previously identified as *D. africana* here is clustered with both *D. africana* and *D. mutila* as well.

Pathogenicity on detached and attached avocado shoots

Lesion length data on detached shoots of Hass and GEM, recorded 2 weeks after laboratory inoculations, are summarized in Figure 1A, respectively. The results of the ANOVA showed that there was a significant effect of cultivar ($P = 0.0019$) and fungal isolate ($P < 0.0001$) on twig lesion length, but the interaction cultivar × isolate treatment was not significant ($P = 0.8395$). All Botryosphaeriaceae species were pathogenic on wounded branches from both cultivars, producing typical canker lesions. Mean lesion length (7.03 ± 6.12 cm) on GEM was significantly larger than that (5.52 ± 5.36) observed on Hass. Significant differences in twig lesion lengths were observed among isolates, with lesions caused by *L. theobromae* (Lth-HA37) and *N. nonquaesitum* (Nnq-HA1, Nnq-HA27) isolates longer than those caused by *N. mediterraneum* (Nm-HA60), *N. australe* (Na-HA19), and the other Botryosphaeriaceae isolates (Fig. 1A). In contrast, lesions caused by *Colletotrichum* (Cf-HA87, Cg-HA94) and *Phomopsis* (Phf-HA5) isolates were significantly shorter (Fig. 1A). There were no lesions observed for uninoculated agar plugs (control). The pathogenicity was confirmed via successful re-isolation from the infected twigs.

Lesion lengths on wounded, attached branches of potted Hass avocado trees are presented in Figure 1B. All Botryosphaeriaceae species were pathogenic on the wounded avocado branches, producing typical canker lesions 2 months after inoculation. Significant differences in internal lesion lengths were noted among isolates ($P < 0.0001$) (Fig. 1B), with lesions caused by *N. nonquaesitum* (Nnq-HA1), *L. theobromae* (Lth-HA37), *N. australe* (Na-HA19), and *N. luteum* (Nl-HA46) isolates longer than those caused by *B. dothidea* (Bdot-HA51), *N. mediterraneum* (Nm-HA60), and *Colletotrichum* (Cf-HA87, Cg-HA94, and Cfio-PL12D46) isolates (Fig. 1B). Mean lesion lengths for the later isolates (Cf-HA87, Cg-HA94, and Cfio-PL12D46) showed no differences from that obtained for the control (agar plugs) (Fig. 1B). The pathogenicity was confirmed via successful re-isolation from the infected branches.

Pathogenicity on attached shoots under field conditions

Lesion lengths on Hass and Lamb Hass avocado shoots, recorded 2 months after field inoculations, are presented in Figure 1C. The results of the ANOVA showed no significant effect of cultivar ($P = 0.434$), with mean lesion length (4.14 ± 1.75 cm) on Hass being similar to that observed (4.35 ± 2.01) on Lamb Hass. All tested isolates induced internal vascular lesions on the inoculated shoots, with smaller lesions recorded on non-inoculated controls. Significant differences in twig lesion lengths ($P < 0.0001$) were observed among isolates. *Lasiodiplodia theobromae* (Lth-HA37), *B. dothidea* (Bdot-HA51), and *Colletotrichum* (Cf-HA87 and Cg-HA94) isolates caused larger lesions on Hass, which were followed by those caused by *N. mediterraneum* (Nm-HA60), *N. nonquaesitum* (Nnq-HA1), *N. luteum* (Nl-HA46), and *N. australe* (Na-HA19) isolates (Fig. 1C). On Lamb Hass, larger lesions were caused by *L. theobromae*, *N. nonquaesitum*, and *N. luteum* isolates, which were followed by those caused by *N. mediterraneum*, *B. dothidea*, and *Colletotrichum* (Cf-HA87) isolates (Fig. 1C). The pathogenicity was confirmed via successful re-isolation from the infected shoots.

Cultivar susceptibility

Inoculations of detached shoots of Hass, GEM, and unreleased avocado cultivars (codes 1 to 7) with *L. theobromae* showed that internal and external lesions developed in all wounded shoots but the controls. There were significant differences in both lesion lengths among cultivars ($P < 0.0001$) (Fig. 2A and B). Unreleased cultivars (codes 2, 3, and 4) had similar internal lesion lengths and appeared to be the most susceptible. Hass displayed the smallest internal lesion length, being less susceptible than GEM. All remaining new

TABLE 5. Distribution of Botryosphaeriaceae species on avocado tissues sampled in different orchards in California

		Count by cultivar		Count by organs				
Species	Total number recovered	Hass	Lamb Hass	Woody tissue	Leaf	Flower	Fresh fruit	Dry fruit
Botryosphaeria dothidea	12	12	–	10	–	2	–	–
Diplodia africana	1	1	–	1	–	–	–	–
Diplodia corticola	1	1	–	1	–	–	–	–
Diplodia mutila	1	1	–	0	1	–	–	–
Diplodia pseudoseriata	1	1	–	1	–	–	–	–
Diplodia seriata	1	1	–	1	–	–	–	–
Dothiorella plurivora	1	1	–	1	–	–	–	–
Lasiodiplodia theobromae	15	15	–	10	3	1	–	–
Lasiodiplodia citricola	4	4	–	4	–	–	–	–
Neofusicoccum australe	2	2	–	2	–	–	–	–
Neofusicoccum mediterraneum	9	9	–	6	1	1	1	–
Neofusicoccum nonquaesitum	15	15	–	15	–	–	–	–
Neofusicoccum australe	30	28	2	21	6	1	2	–
Neofusicoccum luteum	56	46	10	40	9	5	2	–
Neofusicoccum parvum	24	24	–	11	9	1	1	2

cultivars were at least as susceptible as GEM (Fig. 2A). Similar patterns were observed when external lesion lengths were measured for the same cultivars (Fig. 2B), with cultivars of codes 3 and 4 being the most susceptible and Hass the least susceptible.

Inoculation of detached Hass avocado fruit

Both *L. theobromae* (Lth-HA37) and *N. nonquaesitum* (Nnq-HA1) isolates caused circular lesions on the inoculated avocado fruits 10 days after incubation. Significant differences ($P < 0.05$) in lesion length were observed among isolates, with *L. theobromae* producing the largest lesion (Fig. 3). Koch's postulates were fulfilled by re-isolating each fungus from the inoculated fruits.

Colonization of wounded avocado branches following monthly inoculations with mycelial plugs of *L. theobromae* and *N. nonquaesitum*

The seasonal colonization of wounded Hass and Lamb Hass green and lignified avocado branches by *L. theobromae* and *N. nonquaesitum* was monitored in relation to the avocado phenology and climatic conditions and expressed as canker lesion length (Fig. 4A, B, C, and D) and disease incidence.

The ANOVA for data for green shoots showed that infection occurred throughout the sampling periods, with 100% disease incidences. Months were significantly different for canker lesion size ($P < 0.00001$) on both cultivars. Overall, the canker lesions on Hass and Lamb Hass green branches increased significantly in

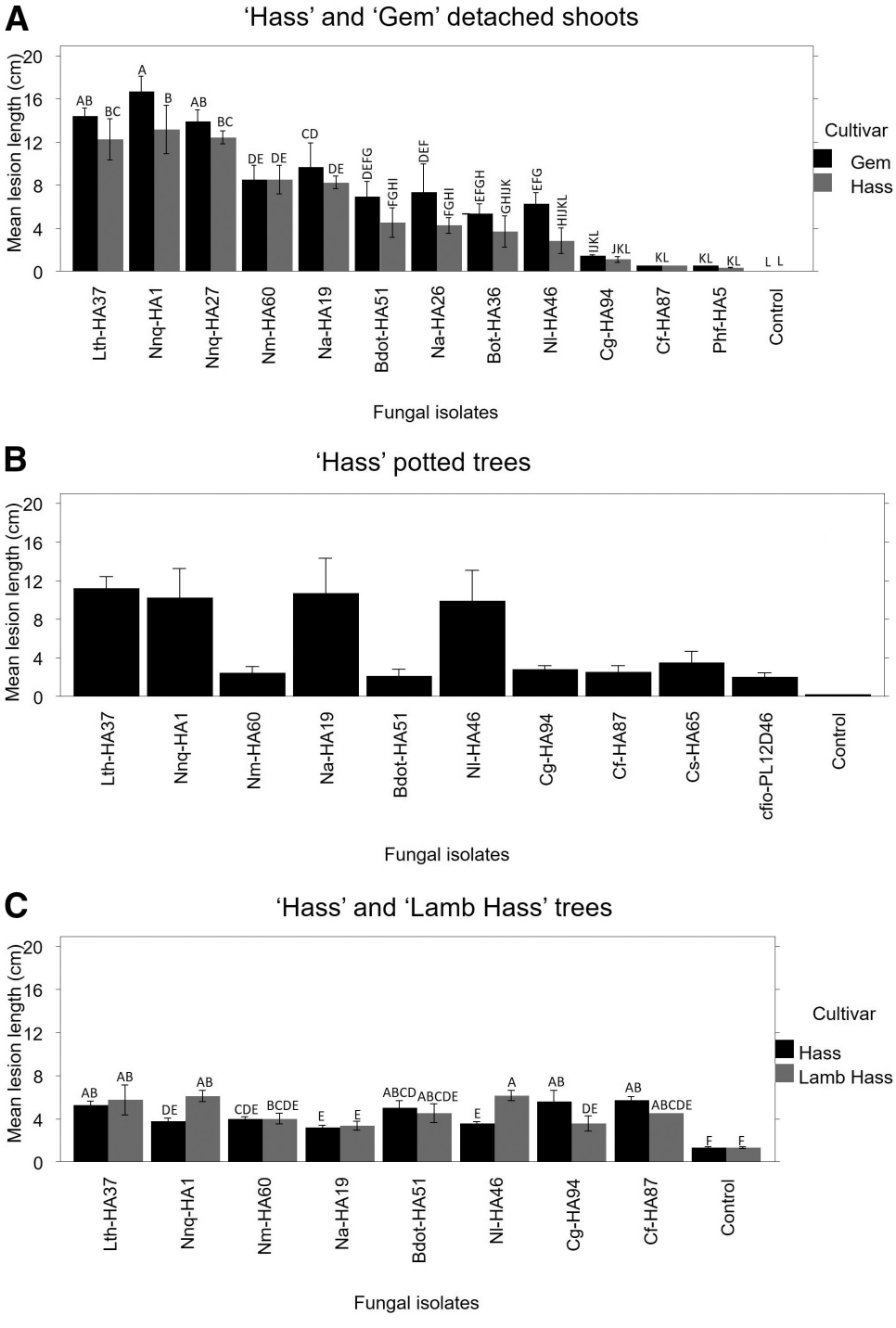

Fig. 1. Mean lesion length (cm) caused by *Botryosphaeria*, *Colletotrichum*, and/or *Phomopsis* species on **A,** detached 'Hass' and 'GEM' avocado twigs, **B,** potted 'Hass' avocado trees, and **C,** 'Hass' and 'Lamb Hass' avocado trees 2 weeks, 2 months, and 2 months after inoculations in laboratory, lathhouse, and field conditions, respectively. Bar graphs represent the means of lesion length values, and vertical bars represent standard errors. Bars with the same letter are not significantly different ($P \geq 0.05$) using the Fisher least significant difference test.

length during the August−October and October−December periods compared with canker lesion lengths recorded in May−July, June−August, July−April, and September−November (Fig. 4A and B). There were significant differences between isolate/control treatments ($P < 0.00001$), and the interactions of month with isolate were also significant ($P < 0.00001$). ANOVA for data for the lignified shoots showed that infection occurred throughout the sampling periods, with 100% disease incidences. Months were significantly different for canker lesion size ($P < 0.00001$). Overall, the canker lesions on Lamb Hass lignified branches did not increase significantly in length during January−April compared with the canker lesion length observed in December 2019. However, the longest lesion lengths were observed in May−July (Fig. 4D). Canker lesions on Hass lignified branches did not increase significantly in length during January−April compared with the canker lesion length observed in December 2019. However, the longest lesion lengths were observed during the May−July periods (Fig. 4C). There were significant differences between isolate/control treatments ($P < 0.00001$), and the interaction of month with isolate was significant for Lamb Hass ($P < 0.00001$).

Colonization of Hass and Lamb Hass avocado branches by the two Botryosphaeriaceae species occurred regardless of the avocado phenology (UCANR 2008).

Effects of temperature and perseitol on radial growth and fungal colonization

The ANOVA results showed that there was a significant effect of isolate ($P < 0.00001$), temperature ($P < 0.00001$), and the interaction of isolate × temperature ($P < 0.00001$) on radial growth of the Botryosphaeriaceae species. The optimal temperature ranged between 25 and 30°C for *L. theobromae*, and it was 25°C for the two other species. Culture diameters were the smallest at 10°C (Fig. 5). All three Botryosphaeriaceae fungi could grow at temperatures between 15 and 30°C, with significantly higher radial growth diameters observed for *L. theobromae* compared with *N. australe* and *N. nonquaesitum* (Fig. 5). Only the *L. theobromae* isolate (Lth-HA37) grew significantly at 35°C, whereas *N. nonquaesitum* (Nnq-HA1) and *N. australe* (Na-HA38) isolates were significantly reduced at this temperature (Fig. 5).

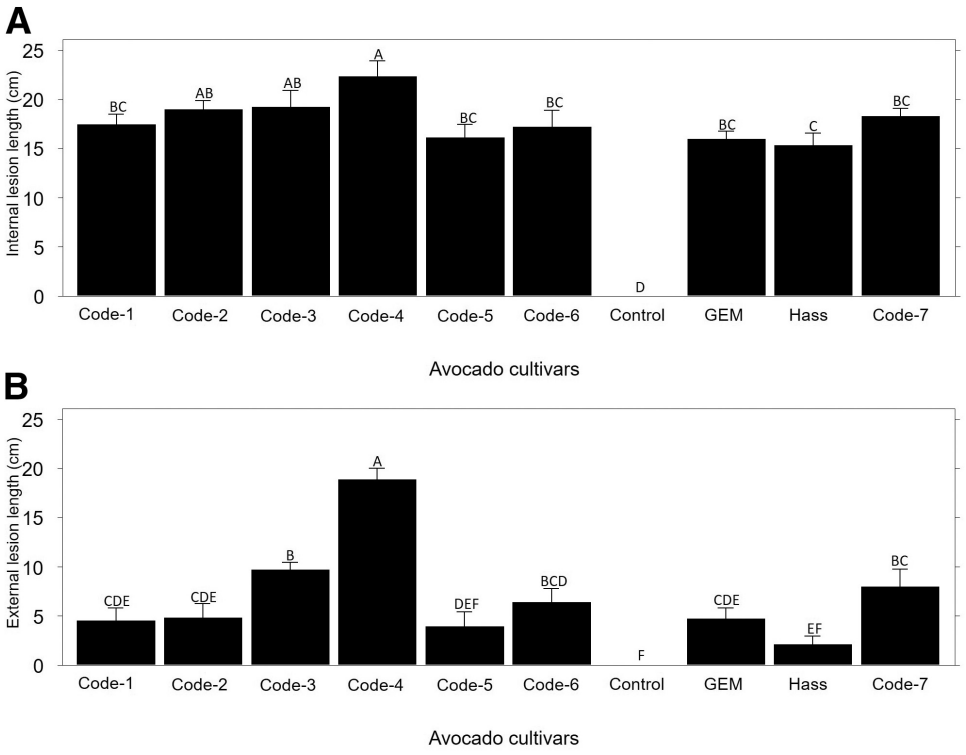

Fig. 2. A, Internal and **B,** external canker lesions in detached twigs of 'Hass', 'GEM', and several unreleased avocado cultivars (codes 1 to 7), measured 2 weeks after inoculations with mycelial plugs of *Lasiodiplodia theobromae* (Lth-HA37). Bar graphs represent the means of lesion length values, and vertical bars represent standard errors. Bars with the same letter are not significantly different ($P \geq 0.05$) using the Fisher least significant difference test.

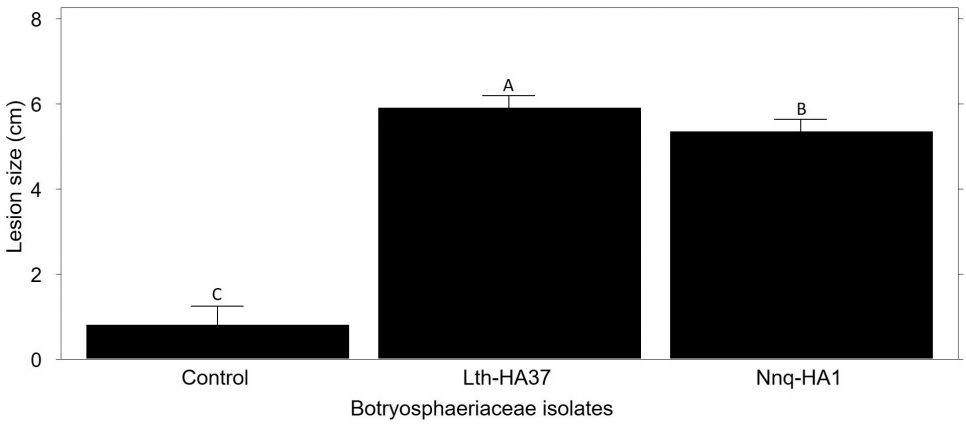

Fig. 3. Lesion formation on detached avocado 'Hass' fruits following inoculations with mycelial plugs of *Lasiodiplodia theobromae* (Lth-HA37), *Neofusicoccum nonquaesitum* (Nnq-HA1), and control agar plugs. The average of lesion length was derived from measurements 10 days after inoculation. Bar graphs represent the means of lesion length values, and vertical bars represent standard errors. Bars with the same letter are not significantly different ($P \geq 0.05$) using the Fisher least significant difference test.

Results of the effect of perseitol on the growth of Botryosphaeriaceae isolates are presented in Supplementary Figure S2. There were no significant differences ($P < 0.001$) in the effect of perseitol concentration on the grow rate of each pathogen; thus, data for all concentrations were combined. Significant differences ($P < 0.0001$) in growth rates were observed among isolates (Supplementary Fig. S2). Radial growth was largest for *L. theobromae* (Lth-HA53 and Lth-HA37) and *N. nonquaesitum* (Nnq-HA1) isolates, followed by those of *N. australe* (Na-V4 and Na-HA19) and *B. dothidea* (Bdot-HA52) isolates. All the other isolates showed significantly lower growths (Supplementary Fig. S2).

Results of the effect of temperature on colonization of Hass shoots by two Botryosphaeriaceae species are presented in Figure 6A (external lesion) and B (internal lesion). The ANOVA results showed that there was a significant effect of isolate ($P < 0.05$) on external lesion length, but temperature and the interaction of

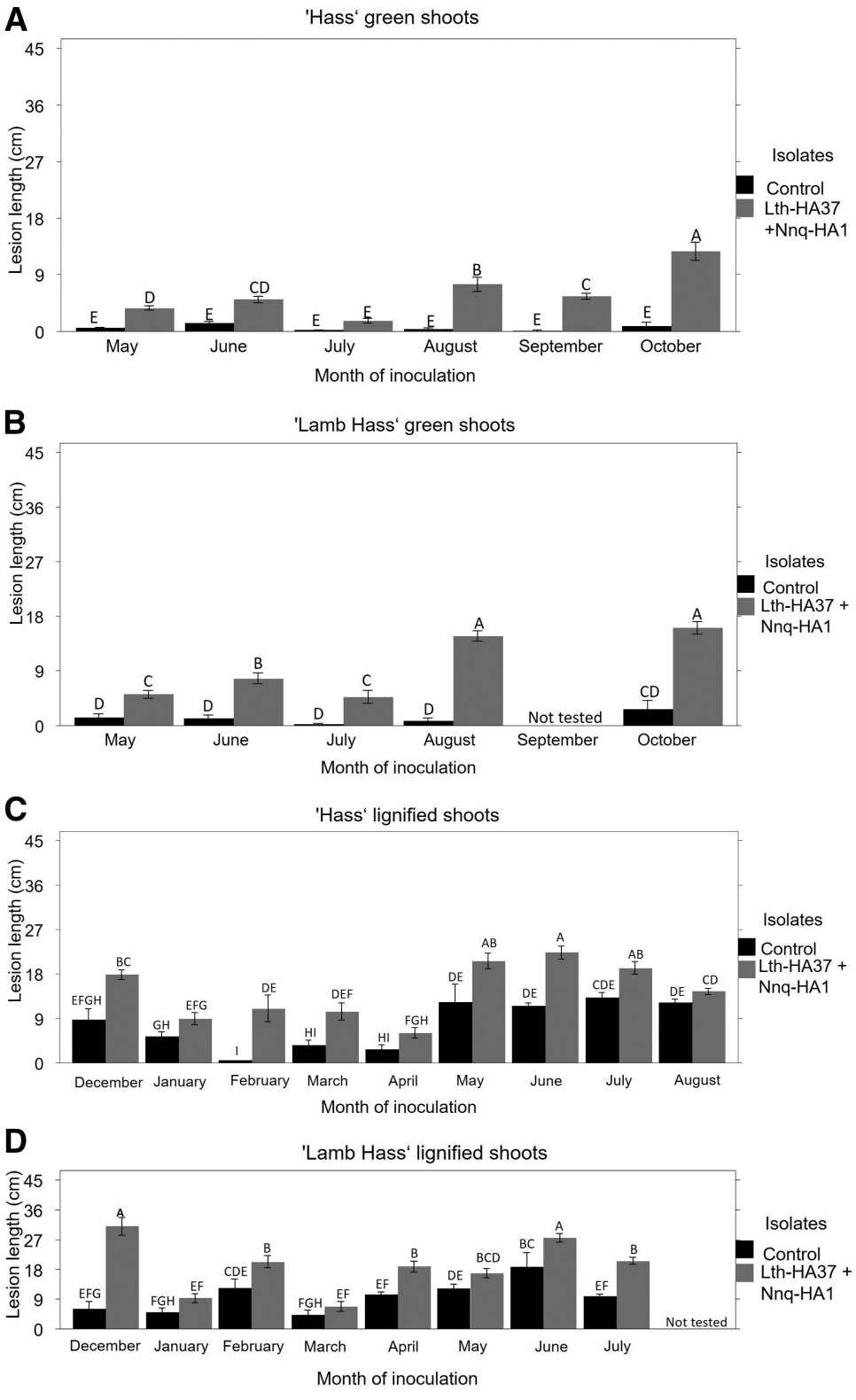

Fig. 4. Canker lesions recorded following inoculations of **A,** 'Hass' and **B,** 'Lamb Hass' green branches and of **C,** 'Hass' and **D,** 'Lamb Hass' lignified branches. Inoculations were performed after wounding at the beginning of each month with mycelial plugs of *Lasiodiplodia theobromae* (Lth-HA37) and *Neofusicoccum nonquasitum* (Nnq-HA1) isolates and internal wood lesions measured at 2- (green shoots) and 4-month (lignified shoots) intervals. Control branches were inoculated with agar plugs. Bar graphs represent the means of lesion length values, and vertical bars represent standard errors. Bars with the same letter are not significantly different ($P \geq 0.05$) using the Fisher least significant difference test.

isolate × temperature were not significant ($P > 0.05$). External lesion length caused by *L. theobromae* was significantly higher at 35°C compared with that caused by *B. dothidea* (Fig. 6A). Additionally, there was a significant effect of isolate ($P < 0.05$) on internal lesion length, but temperature and the interaction of isolate × temperature were not significant ($P > 0.05$). Internal lesion length caused by *L. theobromae* was significantly higher at 25°C compared with that caused by *B. dothidea* (Fig. 6B). Fungal isolations from the inoculated segments showed 100% recovery.

Discussion

Through a series of orchard surveys and laboratory and field experiments, this study presents significant new findings on the occurrence and infection of avocado in a large sampling in avocado-growing areas of California, with identification of the fungal species involved and their pathogenicity on Hass and new avocado cultivars, such as GEM and Lamb Hass. Our fungal isolations and identifications confirmed that common Botryosphaeriaceae fungi, including *Neofusicoccum* spp., *Botryosphaeria dothidea*, and *Lasiodiplodia theobromae*, are well established across the main avocado-producing counties of California. These results are consistent with records from a 2009 survey of Hass avocado orchards in California and other recent surveys of avocado-growing regions in the world, which found high incidences of ABC, dieback, and stem end rot, and Botryosphaeriaceae spp. as the most common fungal species recovered from symptomatic wood and fruit samples (Arjona-Girona et al. 2019; Eskalen et al. 2013; Guarnaccia et al. 2016; McDonald and Eskalen 2011; McDonald et al. 2009; Twizeyimana et al. 2013; Valencia et al. 2019). Our survey further showed that not only do species of Botryosphaeriaceae produce disease symptoms on Hass avocado, but new commercial varieties such as GEM and Lamb Hass and some unreleased cultivars can also be infected by these pathogens. Our pathogenicity experiments in laboratory, lathhouse, and field conditions confirmed that Botryosphaeriaceae inoculations consistently caused severe infection on wounded,

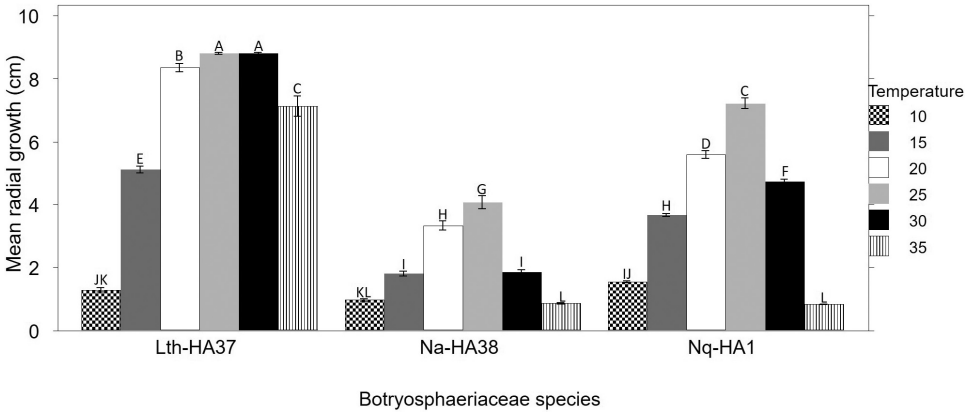

Fig. 5. Effects of temperature on the average mycelial growth (cm) of *Lasiodiplodia theobromae* (Lth-HA37), *Neofusicoccum australe* (Na-HA38), and *N. nonquaesitum* (Nnq-HA1) isolates. The average of the mycelial growth was derived from measurements of the colony diameter on the third day after inoculation on acidified potato dextrose agar. Bar graphs represent the means of radial growth values, and vertical bars represent standard errors. Bars with the same letter are not significantly different ($P \geq 0.05$) using the Fisher least significant difference test.

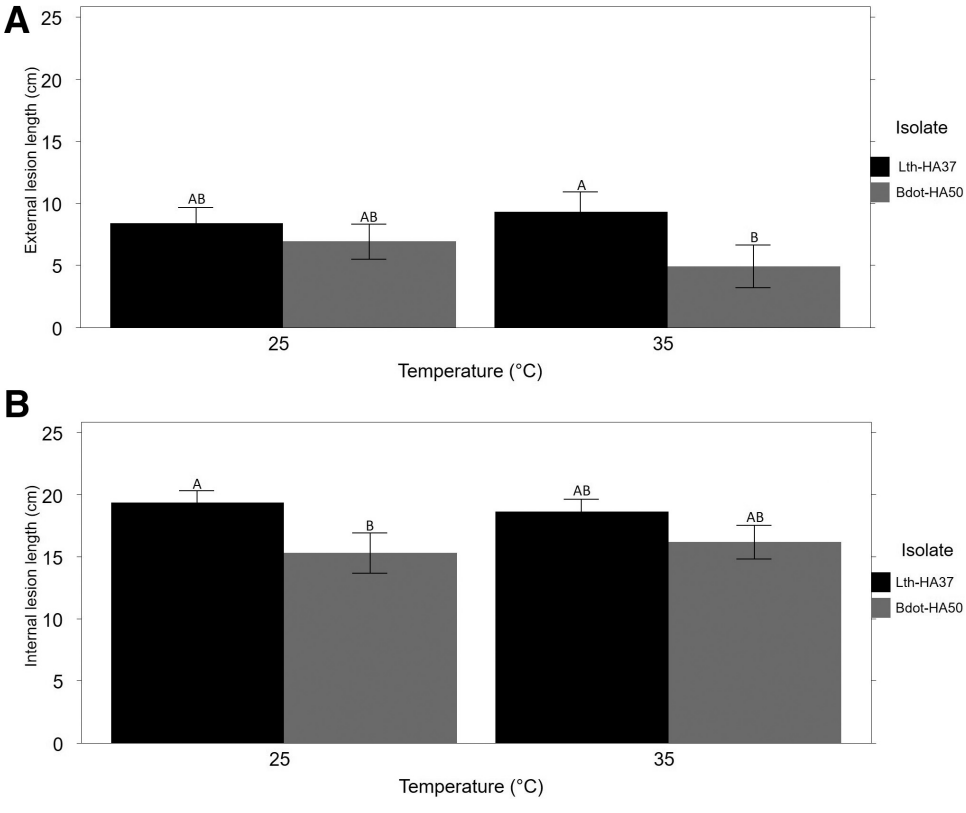

Fig. 6. Development of **A,** external and **B,** internal canker lesions on detached avocado shoots maintained at two temperature regimes 2 weeks after inoculation with *Lasiodiplodia theobromae* (Lth-HA37) and *Botryosphaeria dothidea* (Bdot-HA50) isolates. Bar graphs represent the means of lesion length values, and vertical bars represent standard errors. Bars with the same letter are not significantly different ($P \geq 0.05$) using the Fisher least significant difference test.

detached shoots and fruits, as well as attached shoots on standing trees of various commercial and unreleased avocado cultivars. Previous pathogenicity studies indicated that the Botryosphaeriaceae from Californian and Chilean orchards were pathogenic and could cause branch canker and dieback on Hass avocado (Dann et al. 2013; Guarnaccia et al. 2016, 2020; McDonald and Eskalen 2011; Menge and Ploetz 2003; Twizeyimana et al. 2013; Valencia et al. 2019). Our study is the first to report infection of new avocado cultivars by Botryosphaeriaceae fungi. Similarly, in grapevine, no varietal susceptibility differences were found on excised green shoots of several cultivars inoculated with isolates of several species of Botryosphaeriaceae (Amponsah et al. 2011). In pathogenicity experiments on detached and wounded avocado twigs, it was found that 2 weeks after inoculation, selected Botryosphaeriaceae species were pathogenic on Hass and GEM shoots, with isolates of *L. theobromae* and *N. nonquaesitum* being the most aggressive. This is consistent with reports from other studies showing *Lasiodiplodia* and *Neofusicoccum* species, including *N. nonquaesitum*, *N. parvum*, and *L. pseudotheobromae*, as the most virulent species, causing severe damage in the inner vascular tissue of the stem of plant species such as almond, pistachio, grapevine, and eucalyptus (Inderbitzin et al. 2010; López-Moral et al. 2020; Slippers and Wingfield 2007; Van Niekerk et al. 2004). The predominance of Botryosphaeriaceae in infected branches/twigs and their competitiveness, as proved in the virulence tests, pointed out their role as the main pathological factor involved in ABC formation. Other fungal species of the genera *Colletotrichum*, *Fusarium*, and *Phomopsis* were isolated at lower proportions from the avocado cankered tissues and produced significantly less damage after inoculations on detached shoots compared with lesions caused by *Botryosphaeria* spp., suggesting their potential role as secondary invaders of the xylem tissues, which can sometimes compete with the species of Botryosphaeriaceae under certain, but presently unknown, conditions.

Our random analyses of infected and/or dead branches revealed the presence of spore-producing pycnidia and ascospores-containing pseudothecia. In many host tissues, species of Botryosphaeriaceae have been found overwintering on dead shoots, in diseased wood, and on pruning debris on the orchard floor mainly, on which the canker fungi produce easily and abundantly the above structures (Moral et al. 2019). Both structures have been reported as the main inoculum sources for infection and disease development in the field (McDonald and Eskalen 2011; Moral et al. 2019). In California avocado, pruning debris and leaf litter are often left and mulched on the orchard floor (Gregoriou and Rajkumar 1984). As the development of Botryosphaeria canker is strongly influenced by the number of conidia released in the orchard (Eskalen et al. 2013; Michailides and Morgan 1993), the potential for disease is increased when dead, infected, and cankered tissues bearing fruiting structures are left in the orchard.

Although most Botryosphaeriaceae were recovered from cankered twigs and branches, some were also isolated from naturally infected tissues other than wood (i.e., leaves, fruits, flowers), indicating that colonization of all avocado tissues can occur. Moreover, our monitoring of avocado trees showed that Botryosphaeriaceae could also be isolated from naturally infected but asymptomatic tissues (twig, leave, fruit) and thus can exist latently on avocado tissues. These findings are consistent with results from other studies, which showed that Botryosphaeriaceae are localized in the living and dead branches, twigs, and other organs of avocado trees and could infect fruit and cause both stem end rot and branch canker of avocados (Guarnaccia et al. 2016; Hartill and Everett 2002; Slippers and Wingfield 2007; Twizeyimana et al. 2013). Likewise, a recent study by Reis et al. (2020) found that early symptoms on grapevine green shoots, flowers, and inflorescences were caused by three Botryosphaeriaceae species.

In vitro mycelial growth at different temperatures and in field experiments showed that infection can occur on both green and lignified shoots throughout wounds, regardless of the prevailing weather conditions. Our field inoculations of green and lignified branches showed that Botryosphaeriaceae species were pathogenic on avocado branches of two different phenological stages of the two tested cultivars, Hass and Lamb Hass, actively colonizing the vascular tissues following inoculations of the wounded green and lignified branches across the entire seasons. Notably, these pathogenicity studies emphasized the importance of wounding as the main physical factor enabling these canker-causing fungi to penetrate the avocado host, thus initiating avocado infection by Botryosphaeriaceae pathogens (Michailides 1991; Phillips 1998). Our findings showing the ability of Botryosphaeriaceae isolates to infect avocado shoots of different phenological stages agree with previous studies that showed that severe symptoms are often associated with different types of wounds on lignified and green tissues corresponding to different stages of phenological development (Michailides 1991; Shafi et al. 2019; Van Niekerk et al. 2004). For instance, several studies on grapevine have indicated that several Botryosphaeriaceae isolated from various cultivars of *V. vinifera* were pathogenic not only on mature grapevine wounded wood but also on green shoots and berries, with their virulence ranging from weak to strong (Shafi et al. 2019; Úrbez-Torres et al. 2008; Van Niekerk et al. 2004).

Our temperature-growth relationship study showed that the Botryosphaeriaceae pathogens could grow under a range of temperatures, with an optimum between 20 and 30°C. Although the in vitro growth rate of the pathogens under laboratory conditions might not mimic the actual growth/survival of the pathogen in the plant tissues under a natural environment, this information could contribute to our understanding of the activity and possible infection period of the pathogens. Consistent with results from in vitro growth studies, our field inoculations revealed that colonization/infection of avocado xylem tissues can occur via wounding under various ambient temperatures during the growing season. This is in accordance with previous results that showed that these diseases can develop in different agroclimatic zones of California (McDonald and Eskalen 2011; Twizeyimana et al. 2013), Chile (Valencia et al. 2019), and Italy (Guarnaccia et al. 2016). Likewise, infections in olives, pistachios, and walnuts can occur at any time during the season if there is splashing water and suitable temperatures (10 to 35°C) to induce spore germination (Michailides and Morgan 1993; Moral et al. 2019). Moreover, our inoculation studies showed that whereas *B. dothidea*- and *L. theobromae*-inoculated twigs produced similar internal colonization lengths when incubated at 35°C, external lesions remained significantly longer in *L. theobromae*-inoculated twigs. Because only one isolate was used for each species, the results could not extend to the difference between the two species. The optimal temperature was 25 to 30°C for both Botryosphaeriaceae species, but growth of *L. theobromae* remained significant at higher temperatures of 30 and 35°. *L. theobromae* and *N. nonquaesitum* also grew significantly more than the other Botryosphaeriaceae and fungal species on perseitol-amended media. Because no significant differences in the effect of perseitol concentration on the growth rate of each pathogen were found, the difference in growth rate has nothing to do with perseitol but could be due to a stronger capacity in metabolizing the avocado-produced sugar. The fast-growing rate of *L. theobromae* observed at most of the tested temperatures agrees with other studies (Jacobs and Rehner 1998; Úrbez-Torres et al. 2006; Valencia et al. 2019). The ability of *L. theobromae* to remain virulent and to continue to grow when incubated at higher temperatures indicates that this species is well adapted to high summer temperatures. Similar results have been obtained in other studies in which isolates of *L. theobromae* were much more aggressive at the incubation temperatures of 30 and 35°C in grapevine cane pathogenicity trials (Qiu et al. 2016; Úrbez-Torres et al. 2008). Our results seem to indicate a stronger virulence and greater threat for avocado groves under hot conditions for *L. theobromae*. However, the virulence and prevalence of *Neofusicoccum* isolates as reported in the survey and the fact that high temperatures observed in avocado-growing areas of California can accentuate symptom

expression by all Botryosphaeriaceae indicate that all species in this family would likely be problematic for the health of avocado trees in California.

In summary, our study demonstrates that avocado branch canker and dieback remains prevalent in California avocado-growing regions and provides additional knowledge on Botryosphaeriaceae pathogenic lifecycle on avocados. Our survey provides an updated picture of the general health of Hass and other avocado varieties planted in avocado-growing regions of California, which will be necessary to tailor appropriate and efficient disease management strategies to reduce inoculum sources of pathogens and prevent infections. Our study showed high and moderate frequencies of *Botryosphaeria* fungi in the surveyed traditional and one high-density orchard, respectively. High-density planting, where the spacing between trees is decreased from 6 × 6 to 3 × 3 m, is one strategy that increases yield per acre (Melban 2011). High-density planting requires more intensive canopy management, such as more frequent pruning to manage tree growth. This could lead to an increase in both the number of pruning wounds and the risk of Botryosphaeriaceae infection through pruning wounds if not treated properly, even in orchards with low levels of these pathogens (Dann et al. 2013; Delgado-Cerrone et al. 2016; Eskalen et al. 2013; McDonald and Eskalen 2011). As high-density planting is becoming a common practice in California avocados, Botryosphaeriaceae incidence in such orchards will need to be monitored in future studies. In many perennial specialty crops, including citrus, pistachio, walnut, grapevine, and almond, Botryosphaeriaceae pathogens have been reported to be an increasing economic problem, causing damaging effects to their health and longevity (Eskalen et al. 2013; Mehl et al. 2013; Moral et al. 2019). In the long term, Botryosphaeriaceae prevalence in avocado orchards could become problematic for the avocado industry, considering the absence of registered fungicides for treating pruning wounds and the difficulty of controlling these fungi once inside the plant.

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Literature Cited

Ahimera, N., Gisler, S., Morgan, D. P., and Michailides, T. J. 2004. Effects of single-drop impactions and natural and simulated rains on the dispersal of *Botryosphaeria dothidea* conidia. Phytopathology 94: 1189-1197.

Altendorf, S. 2019. Major tropical fruits market review 2018. FAO, Rome, Italy.

Amponsah, N. T., Jones, E. E., Ridgway, H. J., and Jaspers, M. V. 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.

Amponsah, N. T., Jones, E. E., Ridgway, H. J., and Jaspers, M. V. 2014. Factors affecting *Neofusicoccum luteum* infection and disease progression in grapevines. Australas. Plant Pathol. 43:547-556.

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

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

Bergh, B. O., and Lahav, E. 1996. Avocados. Pages 113-166 in: Fruit Breeding, vol. 1: Tree and Tropical Fruits. J. Janick and J. N. Moore, eds. John Wiley and Sons, New York, NY.

California Avocado Commission. 2020. Industry statistical data. https://www.californiaavocadogrowers.com/industry/industry-statistical-data

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

Carrillo, J. D., Eskalen, A., Rooney-Latham, S., and Scheck, H. J. 2016. First report of *Neofusicoccum nonquaesitum* causing branch canker and dieback of avocado in California. Plant Dis. 100:1778.

Chanderbali, A. S., Soltis, D. E., Soltis, P. S., and Wolstenholme, B. N. 2013. Taxonomy and botany. Pages 31-50 in: The Avocado: Botany, Production and Uses, 2nd Edition. B. Schaffer, B.N. Wolstenholme, and A.W. Whiley, eds. CABI International Press, Wallingford, U.K.

Chen, S., Morgan, D. P., Hasey, J. K., Anderson, K., and Michailides, T. J. 2014. Phylogeny, morphology, distribution, and pathogenicity of Botryosphaeriaceae and Diaporthaceae from English walnut in California. Plant Dis. 98:636-652.

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

Dann, E. K., Ploetz, R. C., Coates, L. M., and Pegg, K. G. 2013. Foliar, fruit and soilborne diseases. Pages 380-422 in: The Avocado: Botany, Production and Uses. B. Schaffer, B.N. Wolstenholme, and A. W. Whiley, eds. CABI International Press, Wallingford, U.K.

Delgado-Cerrone, L., Mondino-Hintz, P., and Alaniz-Ferro, S. 2016. Botryosphariaceae species associated with stem canker, die-back and fruit rot on apple in Uruguay. Eur. J. Plant Pathol. 146:637-655.

Eskalen, A., Faber, B., and Bianchi, M. 2013. Spore trapping and pathogenicity of fungi in the Botryosphaeriaceae and Diaporthaceae associated with avocado branch canker in California. Plant Dis. 97:329-332.

FAOSTAT. 2019. FAOSTAT website. Food and Agriculture Organization Statistics. Rome, Italy.

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

Gregoriou, C., and Rajkumar, D. 1984. Effects of irrigation and mulching on shoot and root growth of avocado (*Persea americana* Mill.) and mango (*Mangifera indica* L.). J. Hort. Sci. 59:109-117.

Guarnaccia, V., Polizzi, G., Papadantonakis, N., and Gullino, M. L. 2020. *Neofusicoccum* species causing branch cankers on avocado in Crete (Greece). J. Plant Pathol. 102:1251-1255.

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

Hartill, W. F. T., and Everett, K. R. 2002. Inoculum sources and infection pathways of pathogens causing stem-end rots of 'Hass' avocado (*Persea americana*). N. Z. J. Crop Hortic. Sci. 30:249-260.

Inderbitzin, P., Bostock, R. M., Trouillas, F. P., and Michailides, T. J. 2010. A six locus phylogeny reveals high species diversity in *Botryosphaeriaceae* from California almond. Mycologia 102:1350-1368.

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

Li, P., Liu, W., Zhang, Y., Xing, J. Li, J. Feng, J. Su, X. and Zhao, J. 2019. Fungal canker pathogens trigger carbon starvation by inhibiting carbon metabolism in poplar stems. Sci. Rep. 9:10111.

Liu, X., Robinson, P. W., Madore, M. A., Witney, G. W., and Arpaia, M. L. 1999. 'Hass' avocado carbohydrate fluctuations, II: Fruit growth and ripening. J. Am. Soc. Hortic. Sci. 124:679-681.

López-Moral, A., Raya, M. C., Ruíz-Blancas, C., Medialdea, I., Lovera, M., Arquero, O., Trapero, A., and Agustí-Brisach, C. 2020. Aetiology of branch dieback, panicle and shoot blight of pistachio associated with fungal trunk pathogens in southern Spain. Plant Pathol. 69:1237-1269.

Martin, G. E., and Bergh, B. O. Avocado Tree Named "3-29-5." US Patent US14, 239 P32003.

McDonald, V., and Eskalen, A. 2011. Botryosphaeriaceae species associated with avocado branch cankers in California. Plant Dis. 95:1465-1473.

McDonald, V., Lynch, S., and Eskalen, A. 2009. First report of *Neofusicoccum australe*, *N. luteum*, and *N. parvum* associated with avocado branch canker in California. Plant Dis. 93:967.

Mehl, J. W. M., Slippers, B., Roux, J., and Wingfield, M. J. 2013. Cankers and other diseases caused by the Botryosphaeriaceae. Pages 298-317 in: Infectious Forest Diseases. P. Gonthier and G. Nicolotti, eds. CABI, Boston, MA.

Melban, K. 2011. Issues watch: Maximizing grower returns. Grove 1:16-18.

Menge, J. A., and Ploetz, R. C. 2003. Diseases of avocado. Pages 35-71 in: Diseases of Tropical Fruit Crops. R. C. Ploetz, ed. CABI Publishing, Wallingford, U.K.

Michailides, T. J. 1991. Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. Phytopathology 81:566-573.

Michailides, T. J., and Morgan, D. P. 1993. Spore release by *Botryosphaeria dothidea* in pistachio orchards and disease control by altering the trajectory angle of sprinklers. Phytopathology 83:145-152.

Moral, J., Morgan, D., Trapero, A., and Michailides, T. J. 2019. Ecology and epidemiology of diseases of nut crops and olives caused by Botryosphaeriaceae fungi in California and Spain. Plant Dis. 103:1809-1827.

Newett, S. D. E., Crane, J. H., and Balerdi, C. F. 2002. Cultivars and rootstocks. Pages 161-188 in: Avocado: Botany, Production and Uses. A. W. Whiley, B. Schaffer, and B. N. Wolstenholme, eds. CABI Publishing, Oxon.

Pérez-Jiménez, R. M. 2008. Significant avocado diseases caused by fungi and oomycetes. Eur. J. Plant Sci. Biotechnol. 2:1-24.

Phillips, A. J. L. 1998. *Botryosphaeria dothidea* and other fungi associated with excoriose and dieback of grapevines in Portugal. J. Phytopathol. 146:327-332.

Phoulivong, S., Cai, L., Chen, H., et al. 2010. *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. Fungal Divers. 44:33-43.

Qiu, Y., Steel, C. C., Ash, G. J., and Savocchia, S. 2016. Effects of temperature and water stress on the virulence of *Botryosphaeriaceae* spp. causing dieback of grapevines and their predicted distribution using CLIMEX in Australia. Acta Hortic. 1115:171-182.

Reis, P., Gaspar, A., Alves, A., Fontaine, F., Lourenço, I., Saramago, J., Mota, M., and Rego, C.-L. 2020. Early season symptoms on stem, inflorescences and flowers of grapevine associated with *Botryosphaeriaceae* species. Plants (Basel) 9:1427.

Rolshausen, P. E., Arpaia, M. L., and Faber, B. 2016. High density planting for avocado production: A Chilean perspective. Grove 2016:14-17.

Shafi, A., Ridgway, H. J., Jaspers, M. V., and Jones, E. E. 2019. Factors influencing virulence and conidial production of *Neofusicoccum* species on grapevine shoots. Eur. J. Plant Pathol. 153:1067-1081.

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

Teviotdale, B. L., Michailides, T. J., and Pscheidt, J. W., eds. 2002. Compendium of Nut Crop Diseases in Temperate Zones. American Phytopathological Society, St. Paul, MN.

Trapero, A., Roca, L. F., Trapero, C., López-Escudero, F. J., and Moral, J. 2011. Diseases and their control. Pages 89-135 in: Culture and Knowledge Inspired by Olive Oil. J. Humanes, J. Villar, M. Fialho, and P. Higueras, eds. GEA Westfalia Separator Iberica SA, Ubeda-Jaén, Spain.

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

UCANR. 2008. Integrated Pest Management for Avocados. UCANR Publication 3503.

United States Department of Agriculture, Economic Research Service (USDA ERS). 2020. Fruit and Tree Nut Yearbook Tables. https://www.ers.usda.gov/topics/crops/fruit-and-tree-nuts/

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

Valencia, A. L., Gil, P. M., Latorre, B. A., and Rosales, I. M. 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.

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

Van Niekerk, J. M., Strever, A. E., du Toit, P. G., and Halleen, F. 2011. Influence of water stress on *Botryosphaeriaceae* disease expression in grapevines. Phytopathol. Mediterr. 50:S151-S165.

Zea-Bonilla, T., Gonzalez-Sanchez, M. A., Martin-Sanchez, P. M., and Perez-Jimenez, R. M. 2007. Avocado dieback caused by *Neofusicoccum parvum* in the Andalucía Region, Spain. Plant Dis. 91:1052.