Prevalence, Diversity, Pathogenicity, and Infection Phenology of Botryosphaeriaceae causing Avocado Branch Canker in California.

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Project leader: Themis J. Michailides, Position Title: Plant Pathologist
Address: University of California, Davis, Kearney Agricultural Research & Extension Center, 9240 South Riverbend Ave., Parlier, CA 93648; Primary Telephone Contact Number: 559-646-6546 (mobile 559-351-2569); E-mail Address: tjmichailides@ucanr.edu
Major contributor: Herve Avenot [Project Scientist/UC Davis, Kearney Agric. Research and Extension Center (KARE)]; E-mail Address: hfavenot@ucanr.edu
Other cooperators: Dr. Mary Lu Arpaia (UC Riverside, KARE), James Davis (PCA, San Diego County), Ben Faber (UCCE Ventura and Santa Barbara Counties); Christopher Greer (UCCE San Luis Obispo); California avocado growers and nursery managers.

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. Botryosphaeriaceae were the predominant fungi recovered from cankered tissues collected across the surveyed orchards. These fungi caused symptoms on all sampled cultivars and were detected in asymptomatic twigs and other organs. Characterization of 173 *Botryosphaeria* isolates using molecular methods showed *Neofusicoccum* were the most frequently recovered species from both Hass and Lamb Hass. Pathogenicity tests, on excised ('Hass', 'GEM', and 'Hass' mutants) and attached shoots from potted ('Hass'), and mature avocado trees ('Hass' and 'Lamb Hass') in the field, showed that all tested *Botryosphaeria* species caused canker lesions on wounded, green and mature branches of the specified cultivars. In temperature-dependent growth and infection studies, the growth of the pathogens was higher between 20 and 30 °C, but only *Lasiodiplodia. theobromae* significantly grew and caused external lesions at 35 °C. 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 and ambient air temperature fluctuations. Overall, this study extended our knowledge of Botryosphaeriaceae pathogenic life cycle on avocados, which will be useful to tailor management strategies.

Introduction
Botryosphaeria branch canker and dieback of avocado (*Persea americana* Mill.), commonly referred to as Avocado Branch Canker (ABC), and formerly known as Dothiorella canker, is a fungal disease that currently represents a threat for avocado production worldwide because of the important economic losses resulting from reduced yield of affected trees and their premature death. Over the past several years, avocado growers, private consultants, and extension specialists have all noted that ABC is increasingly common in avocado growing areas of California. A statewide survey of mature avocado orchards, conducted more than 10 years ago, showed widespread occurrence of ABC disease in avocado producing counties of California (McDonald and Eskalen 2011). Furthermore, advances in molecular techniques allowed for more in-depth investigation of the pathogens, revealing a diversity of fungal species within the Botryosphaeriaceae family as the causal agents of this disease (McDonald and Eskalen 2011).

These pathogens can survive as parasites or saprophytes, but many are latent pathogens of woody shrubs and trees, which may live undetected in an asymptomatic until stressful conditions weaken the host and symptoms are expressed (Slippers and Wingfield 2007). By definition, a latent infection involves a parasitic relationship of the pathogen and the host that eventually induces macroscopic symptoms. Moisture stress (drought), as experienced in California in recent years, is associated with an increase in *Botryosphaeria* infection and canker expansion. These fungi overwinter as pycnidia (small dark 'pimple-like' structures) on the surface of diseased wood under the bark (Eskalen et al. 2013). Following hydration during the rainy season, pycnidia release asexual conidia (spores) which are spread by rain splash and wind, disseminating the fungi from tree to tree, and from one part of the tree to another. Although, these fungi can infect a wide range of woody plants through lenticels, ABC mainly develops when conidia land on freshly cut or damaged wood from fresh pruning wounds and other mechanical or sunburn damage. The conidia germinate and invade the woody tissue via xylem vessels and damage the vascular system. Cankers form around the initial infection point. Symptomatic branch cankers exhibit necrotic, friable bark, red-brown cankers and branch dieback associated with characteristic whitish exudate of perseitol while internally, the wood becomes reddish brown. Rarely, these fungi also form flask-shaped sexual fruiting bodies (pseudothecia), almost always intermingled with the pycnidia, on the outside of cankers, producing sexual fungal spores (ascospores) disseminated by wind and rain splash to infect the plant via fresh pruning wounds (Eskalen et al. 2013).

Although previous studies have shed light on the identity, diversity, and pathogenicity of Botryosphaeriaceae species attacking 'Hass' avocado in California (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. Once inside the plant, these fungi are very difficult to control. The absence of registered fungicides against ABC is a serious concern for the California avocado industry. Following a 2018 meeting with the California Avocado Commission (CAC), our plant pathology group at the University of California, Kearney Agricultural Research and Extension (UC, KARE) Center (Parlier, CA), received funding from the Commission to learn more about the prevalence of *Botryosphaeria* species in California avocado groves and their pathogenic life and disease cycles in relation to the avocado phenological stages, cultivar susceptibility, and prevailing weather conditions. During an initial survey in 2017, in addition to *Botryosphaeria*, *Colletotrichum* fungi of the Glomerellaceae family were also recovered in the cankered tissues, but to a lesser degree. The presence of *Colletotrichum* fungi raised the question of whether or not they also play a role in avocado canker formation or simply colonize avocado wood tissues as saprophytes. Historically, one *Colletotrichum* species, namely *C. gloeosporioides*, has been known to occur on avocado and other tropical fruits crops. It is typically a postharvest pathogen that cause avocado fruit rotting, but it also occurs in orchards, both as causal agent of anthracnose on leaves and fruits and of latent infections.

The overall goal of this research was to gain further insights on the etiology and epidemiology of ABC and anthracnose, which are critical to developing and providing disease management recommendations for the industry. Specific objectives during the first year of this research were to: 1) determine the extent of ABC and anthracnose problems in avocado groves, the occurrence of latent infections, and sources of inoculum in mature avocado groves; 2) identify the most aggressive species of fungi ; 3) study the life cycle and disease cycle of these fungi in avocado groves; 4) determine when and how avocado shoots are infected; 5) determine what factors influence disease expression; and 6) determine whether infections remain latent (dormant) but later cause disease symptom expression.

Materials and methods

Survey of old mature avocado groves, plating of sampled avocado tissues, and pathogen isolation and incidence.

Field surveys were conducted in the Fall of 2018 and Spring of 2019 in 22 commercial and experimental groves located throughout California's avocado growing regions. Orchards were located and surveyed in collaboration with a UC horticulture specialist, UC Cooperative Extension Advisors, and Pest Control Advisers in San Diego, Riverside, Ventura, and San Luis Obispo (**Table 1**). The orchards varied in age and consisted of the 'Hass' and 'Lamb Hass'. Samples from 'Gem' and other unreleased cultivars were sampled from an experimental orchard at the UC Lindcove Research and Extension Center (REC) in Tulare County (**Table 1**). Infected wood tissues (necrotic, cankered, or dead branches /twigs) with or without sunburn damages and other symptomatic and symptomless avocado organs (twigs, leaves, fruits), were collected from trees showing characteristic dieback symptoms. Isolations were performed on acidified potato medium by plating sections of necrotic and green, healthy tissue. Growing fungal isolates were subsequently identified at the genus level based on colony and conidial morphology. The incidence of *Botryosphaeria* and *Colletotrichum* and other recovered fungi were then determined.

Identity of the causal pathogens at the species level and pathogenicity on avocado cultivars.

Molecular methods were used to classify the isolated pathogens at the species level. Representative isolates of the identified *Botryosphaeria* and *Colletotrichum* genera were selected for maintenance and mycelia were used for DNA extractions. Species identifications were determined by sequencing of specific genomic regions and comparison with established databases. To determine the most aggressive isolates, pathogenicity of the identified fungal species was assessed on healthy, detached green shoots (one-year-old) of 'Hass' and 'Gem' varieties of avocado and attached shoots of potted Hass trees, and Hass and Lamb trees in the field (Pine Tree Ranch, Santa Paula, CA). Detached and attached shoots were wounded by removing a piece of cambium with a cork-borer and then inoculated by placing agar plugs infested with one isolate of representative fungi onto the wound. Control shoots were inoculated with uninfested agar plugs. Excised shoots were incubated at room temperature (~75.2°F) and under humid conditions for two weeks. Resulting canker lesion lengths were measured 2 weeks (excised shoots) and 2 months (attached shoots) after inoculations. Isolations were made from all inoculated to confirm pathogenicity.

Conditions for pathogen infection in avocados.

To explore the effect of temperature on disease expression and development, the effect of various temperatures on the growth of selected pathogens was assessed in controlled laboratory environments. Mycelial plugs (8 mm) of 3 Botryosphaeriaceae isolates, namely *Lasiodiplodia theobromae* (Lth-HA37), *Neofusicoccum nonquaesitum* (Nnq-HA1), and *Neofusicoccum australe* (Na-HA38) were cut from active PDA cultures and transferred in the center of new PDA plates. These cultures were incubated at six different temperatures (10, 15, 20, 25, 30 and 35°C) in the dark for 3 days. Colony diameters were recorded after 3 days in two orthogonal directions.

Phenology of infection of ABC and factors influencing disease initiation and development. Field experiments at the Pine Tree Ranch (Santa Paula, CA) were carried out to examine *Botryosphaeria* infection and disease progression on avocado shoots and other attached organs in relation to avocado phenological stages. The influences of wounding and environmental conditions on infection at different times of the year were also assessed. Pathogen inoculum availability on green, symptomless tissue (latent infections) was also monitored after surface-disinfestation. Monthly, artificial inoculations were performed with mycelial plugs of two *Botryosphaeria* pathogens (*L. theobromae* and *Neof. nonquaesitum*) or a conidial suspension (*L. theobromae*). Inoculations with mycelial plugs were done on wounded, healthy green (2019) and lignified (2020) branches of 'Hass 'and 'Lamb Hass' cultivars. Inoculations with spore suspensions were done on wounded twigs or non-wounded tissues. Canker lesion lengths on green and/or lignified branches were recorded 2 and 4 months after inoculations, respectively.

The influence of water and heat stress factors on disease initiation and development by Botryosphaeriaceae on avocado were also determined in lathhouse experiments. Initially healthy looking young potted-avocado trees (Gem and Hass on Dusa or Toro Canyon rootstock) were obtained from a commercial nursery and placed near our greenhouse at UC KARE. A set of trees were continually irrigated for about 5 weeks while the other set was not. The non-irrigated trees developed canker and dieback symptoms, apparently through naturally occurring infections, after the applied water stress event, while irrigated plants did not develop any symptoms. Subsequently, pieces of asymptomatic and symptomatic twigs were plated on acidified agar medium followed by isolations of the associated fungi. The effect of water stress was also assessed in two consecutive summers by exposing a set of potted Hass avocado trees to sunlight and 25% irrigation regime (stressed trees) vs. a set of plants placed under shade and subjected to 100% irrigation regime (control plants). Records of canker lesion sizes from both sets were collected about 2 months after inoculation with *L. theobromae*.

Results and discussion.

Survey of old mature avocado groves and determination of pathogen incidence. Analyses of pathogen incidence data from the surveyed orchards showed that Botryosphaeriaceae causing ABC were the fungi predominantly associated with the cankered branches in both sampling years (**Table 2**). These fungi were present in every sampled grove and caused symptoms on all surveyed cultivars, including Hass, Gem, and Lamb Hass (**Table 2**). In addition to woody tissue, these fungi were also detected on other avocado organs, including leaves (**Table 3**), flowers, fruit stems (peduncles), and young and mature fruits (**Table 4**). Moreover, *Botryosphaeria* were also present in symptomless tissues (flowers, twigs, leaves, fruits) (data not shown), thus revealing the presence of latent infections, because isolations are made after a harsh surface sterilization that cleans the host tissue of any propagules of these fungi or other contaminants. In other words, these pathogens are ubiquitous and simply looking for a point of entry (i.e. wound) to enter the tree. In fact, an important observation from this survey was that *Botryosphaeria* were recovered at high frequencies from infected twigs or dead tissues that sustained sunburn damage compared to wood tissue with no sunburn injury (**Table 5**). This type of injury also facilitates pathogen infection and emphasizes the need to protect wood tissue from sunburn. In contrast to *Botryosphaeria*, *Colletotrichum*, the causal agent of anthracnose, was less common in the cankered tissues of both Hass and Lamb Hass, but along with *Alternaria* saprophytic fungi, they were most frequently isolated from symptomatic leaves

(**Tables 2** and **3**). This result indicates that *Colletotrichum* pathogen(s) may act as secondary invaders of avocado xylem tissues. Throughout this survey, numerous fruiting structures that produce asexual and sexual spores of *Botryosphaeria* (pycnidia and pseudothecia) and *Colletotrichum* (acervulli and perithecia) were observed on both living and dead tissues (branches and leaves) from the orchards. This further indicates how these pathogens survive and establish in groves and the difficulty growers will have trying to eliminate these sources of pathogen inocula and infection. Other fungi, including *Fusarium* and *Phomopsis* were also isolated from the samples, but at lower proportions (**Tables 2** and **3**).

Identity of the causal pathogens at the species level and impact on avocado cultivars.
Our DNA analyses of the recovered fungi confirmed that common species (about eleven species) of Botryosphaeriaceae are found in avocado groves throughout California. These include species such as *Lasiodiplodia theobromae*, *Botryosphaeria dothidea*, and *Neofusicoccum*, with the latter being the most prevalent species throughout the sampled areas (**Table 6**). This new information shows that there is no distribution of *Botryosphaeria* according to their geographic origin as reported earlier, but also leads to new questions on how best to manage these pathogens. Our new molecular data showed that avocado anthracnose disease is actually caused by several *Colletotrichum* species within the *C. gloeosporioides* species complex, comprising *C. alienum*, *C. perseae*, *C. siamense*, *C. fructicola*, and *C. gloeosporioides*. All of the six selected species of the Botryosphaeriaceae were found to be pathogenic to Hass and Gem avocados, with *Lasiodiplodia* and *N. nonquaesitum* species being the most virulent (aggressive) (**Figures 1A** and **1B**). All of the six species of the Botryosphaeriaceae were also found to be more aggressive than the *Colletotrichum* and *Phomopsis* species (**Figures 1A** and **1B**). Pathogenicity on wounded branches Hass and Lamb Hass in the field and/or lathhouse showed most isolates caused similar lesions, 2 months after inoculations (**Figure 2**). Furthermore, inoculations of wounded detached shoots of various unreleased avocado cultivars, grown at the Lindcove REC, showed that they are susceptible to *Botryosphaeria* infection (data not shown). Ultimately, these studies confirm the role of the Botryosphaeria pathogens as the primary causal agents of ABC and the role of *Colletotrichum* as secondary invaders of avocado xylem tissues. A possible interaction between the two pathogens remains to be investigated.

Conditions for pathogen infection in avocados.
Botryosphaeria infection can quickly lead to cankers with favorable environmental conditions. Results from growth-rate assay revealed that the pathogens differed in their ability to grow under different temperature regimes. Pathogen growth rates generally increased from 68 °F to 86 °F, and only *Lasiodiplodia* continued growing above 95 °F (**Figure 3**). This indicates that the latter could become more predominant in warmer growing regions.

Phenology of infection of ABC and factors influencing disease initiation and development.
Our monitoring of the weather conditions and occurrence of latent infections in symptomless avocado tissues (natural infections of flowers, twigs, leaves, fruits) showed that the conditions at the Santa Paula site were generally dry. Despite this, *Botryosphaeria* pathogens were detected on avocado tissues collected in the grove, but at a low level. Results from monthly, mycelial plug inoculations on wounded, green and lignified branches showed that both branch types were susceptible to *Botryosphaeria* infections throughout the inoculation and sampling periods and regardless of the avocado phenological stage or ambient air temperature fluctuations (**Figures**

4A and **4B**). Inoculations with spores resulted in the development of canker lesions on wounded branches (**Table 7**). In contrast, non-wounded tissues did not develop any symptoms but exhibited latent infections. Symptoms occurred on twigs, leaves, and developing fruits when humidity conditions were maintained for a long period by covering the tissues with a plastic bag. The fact that infections occurred mainly on wounded shoots or during period of high humidity underscore the importance of wounding. The latter can break the quiescent infections, enhance the infectivity of *Botryosphaeria*, and lead to canker formation while the local dry conditions reduce the chances of successful infections on unwounded tissues.

Plating of pieces of asymptomatic and symptomatic twigs on acidified agar medium followed by isolations of the associated fungi showed that *Botryosphaeria* fungi could be recovered among other pathogens, indicating that they can exist latently in twig tissues prior to exposure to water stress, which can induce the disease. Similarly, results of experiment comparing stressed vs. non stressed avocado trees showed the stressed trees had larger canker lesion sizes compared to the control plants (data not shown).

Conclusions

Our survey of avocado orchards and subsequent fungal isolations and identifications confirmed that common Botryosphaeriaceae fungi, including *Neofusicoccum* spp., *Botryosphaeria dothidea*, and *Lasiodiplodia theobromae,* are well established across the main avocado growing areas of California and the primary fungal pathogens associated with ABC. These results are consistent with records from a 2009 survey of avocado orchards in California and other recent surveys of avocado growing regions in the world which found high incidences of avocado branch canker, 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 et al. 2009; McDonald and Eskalen 2011; Twizeyimana et al. 2013; Valencia et al. 2019). Although most Botryosphaeriaceae were recovered from cankered twigs and branches, some were also isolated from naturally infected tissues other than wood (leaves, fruits, flowers), indicating that colonization of all avocado tissues can occur. In our study, isolation of *Botryosphaeria* could also be made from naturally infected but asymptomatic tissues. This showed that these pathogens can grow in live avocado tissues without causing any apparent symptoms and lends support to the likelihood of latent infections (Slippers and Wingfield 2007). Moreover, our monitoring of artificially-inoculated avocado trees also showed that *Lasiodiplodia* existed latently on avocado tissues (twig, leave, fruit) throughout the inoculation and sampling periods; symptoms did not develop even when many latent infections were present, but they were only seen (associated with high incidence of *Lasiodiplodia* isolation) with increased and persistent humidity condition which was created by keeping the bag covering the tissues for an extended period (data not shown). This study led to a better understanding of the pathogen life and disease cycles as illustrated in **Figure 5**. Pathogen inoculum seems present year-round, and infections can occur throughout the growing season regardless of the phenological stage and ambient temperature, year-round, with wounds being the primary sites of infection. Our random analyses of infected and/or dead branches revealed that the pathogen primary inoculum, namely spore-producing structures pycnidia splashed by rain and irrigation water and pseudothecia releasing wind-borne ascospores, can be present on avocado debris. Adherence to best management practices recommended for the management of canker and dieback pathogens should also be followed for managing ABC in avocado groves. These practices include:

a) avoid pruning during or immediately after rain, dew or heavy fog;
b) prune out dead limbs and twigs that carry the pathogen fruiting structures during dry periods followed by immediate removal of pruning debris from the grove to the extent practical to reduce inoculum levels,
c) sanitizing pruning equipment;
d) properly prune dense canopies to increase air flow and reduce humidity;
e) reduce tree stress and maintain trees in good condition through appropriate irrigation and fertilization practices.

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Table 1. Sampled avocado orchards in California counties of production.

County	City	Orchard		Avocado cv	Date of sampling [1]
		Name	Number sampled		
Ventura	Santa Paula	DA-1	4	'Hass'	November 2018
		LIM high-density			
		LIM-O			
	Fillmore	G3			November 2018
	Pomona	PTR	1 experimental orchard	'Hass'	November 2018
				'Lamb Hass'	April 2019
San Diego	Bonsall	West-L	1	'Lamb Hass'	November 2018
	Bonsall	West-L	1	'Hass'	
	Valley Center	Mesa-C	1	'Hass'	
		ZRT	1	'Hass'	
	Pauma Valley	Starbeam	1	'Lamb Hass'	
	Pauma Valley	Starbeam	1	'Hass'	
	Fallbrook	NIG	1	'Hass'	
Riverside	Riverside	RC1	3	'Hass'	November 2018
		RC2			
		RC3			
San Luis Obispo	Morro Bay	GR1	1	'Hass'	November 2018
	Morro Bay	GR1	1		April 2019
	San Luis Obispo	GR2	1		November 2018
	San Luis Obispo	GR2	1		April 2019
	Morro Bay	GR3	1		
	Cayucos	GR4	1		
Tulare	Exeter	Lindcove experimental orchard	1	'Hass'	November 2018, April 2019
				'Gem'	
				'Carmen'	
				Unreleased avocado cultivars	

[1] Samples collected in November 2018 and April 2019 included symptomatic and asymptomatic twigs or branches (with or without sunburn damage), leaves, peduncles, petioles, mummies, and fruits.

Table 2. Incidence of *Botryosphaeria* and other fungi in cankered avocado branches collected in 2018 and 2019 from avocado orchards in several producing counties of California.

					Pathogen incidence (%) in branch samples				
County	**Orchards**	**Year**	**Cultivar**	**Branch cankers**	***Botryosphaeria***	***Colletotrichum***	***Phomopsis***	***Alternaria***	***Fusarium***
Riverside	RC1	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	PTR	2018	'Hass'	263	62.4	17.9	0	7.2	8.7
		2019	'Lamb Hass'	50	50	4	0	54	0
	G3	2018	'Hass'	104	83.6	8.6	0	0	4.8
	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	West-L	2018	'Hass'	117	79.5	11.1	0	0.8	7.7
	ZRT			42	57.1	16.7	0	0	9.5
	Mesa-C			63	28.6	20.6	3.2	14.3	15.9
	Starbeam			51	19.6	5.9	2	29.4	39.2
	NIG			32	6.2	9.4	0	15.6	68.7
	West-L		'Lamb Hass'	94	67	26.6	0	9.6	14.9
	Starbeam			53	75.5	5.7	0	7.6	15.1
San Luis Obispo	GR1	2018	'Hass'	38	90	14	0	0	0
	GR2			223	81.6	9.4	0	0.9	0.4
	GR1	2019		161	67.1	23.6	9.9	5.6	2.5
	GR3			100	85	5	1	2	6
	GR4			50	24	24	14	16	4
	GR2			163	81.6	7.4	14.1	4.9	9.2
Tulare	Lindcove Station	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
		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
				2565	**43.6**	**13.3**	**4.5**	**19.8**	**10.1**

Table 3. Incidences (%) of *Botryosphaeria* and other fungi in symptomatic avocado leaves collected in 2018 from several avocado orchards in California.

County	Orchards	Cultivar	Infected leaves [b]	Pathogen incidence (%) [a]				
				Botryosphaeria	***Colletotrichum***	***Phomopsis***	***Alternaria***	***Fusarium***
Riverside	RC1	Hass	53	15	13	2	72	8
	RC2		23	0	4	0	48	4
	RC3		53	2	17	0	96	0
Ventura	PTR	Hass	20	0	0	0	80	0
	G3		30	20	23	3	57	0
	LIM High density		81	0	19	0	84	3
	LIM-O		11	27	64	9	0	0
	DA1		120	15	32	0	51	1
San Diego	West-L	Hass	42	41	36	0	14	7
	ZRT		10	0	0	0	100	0
	Starbeam		15	0	0	0	47	53
	NIG		41	7	68	0	22	2
	West-L	Lamb	31	23	90	0	29	0
	Starbeam	Hass	30	0	0	0	93	3
San Luis Obispo	GR1	Hass	52	43	66	0	0	0
	GR2		10	0	0	0	100	0
			Total = 622	**12**	**27**	**1**	**56**	**5**

[a] Leaf samples per orchard yielding indicated fungi. Numbers in bold are averages per orchard.
[b] Number of infected leaves collected in each orchard.

Table 4. Incidence of *Botryosphaeria* spp. in other symptomatic avocado organs and debris collected in 2018 and 2019 from orchards in several producing counties of California.

County	Orchard	Year	Cultivar	***Botryosphaeria* incidence (%) in avocado samples**						
				Infected petioles	Infected peduncles	Infected fruits	Fruit mummies	Infected pedicels	Soil debris	Infected Inflorescences
Riverside	RC1	2018	‘Hass’	25	19.2	14.8	5	-	-	-
	RC2			0	9.5	0	10	-	-	-
	RC3			0	0	0	30	-	-	-
Ventura	PTR	2018	‘Hass’	-	10	-	-	-	-	-
	DA1			39.6	3.5	-	-	-	-	-
	PTR	2019	‘Lamb Hass’	3.5	-	-	10	-	-	5
San Diego	West-L	2018	‘Hass’	-	-	88	-	-	-	-
	NIG			3.3	35.7	-	-	-	-	-
	Starbeam		‘Lamb Hass’	1	-	2.4	1	-	-	-
San Luis Obispo	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

Table 5. Incidence of *Botryosphaeria* on cankered branches with or without sunburn injury.

County	***Botryosphaeria* (%) in cankered branches/twigs**			
	No sunburn		With severe sunburn	
	Range	Mean	Range	Mean
Riverside	20 - 82	53.4±23.9	20 - 100	71.9±35.8
Ventura	5 - 100	60.2±35.6	20 - 100	65.9±25.5
San Diego	10 - 100	50.4±40.4	27.5 - 100	75.2±23.5
San Luis Obispo	55 - 60	57.5±3.5	20 - 100	77.9±26.2

Table 6. Distribution of *Botryosphaeria* species from avocado in Counties of production from 2017 through 2020.

Species	Total # Recovered	Count by cultivar		County			
		Hass	Lamb Hass				
Botryosphaeria dothidea	9	9		San Luis Obispo	Riverside	San Diego	Ventura
2017				1			
2018							4
2019				4			
Lasiodiplodia theobromae	10	10		San Luis Obispo	Riverside	San Diego	Ventura
2017							
2018					1	4	
2019							5
Lasiodiplodia citricola	4	4		San Luis Obispo	Riverside	San Diego	Ventura
2017							
2018					1		1
2019							2
***Lasiodiplodia* spp.**	5	5		San Luis Obispo	Riverside	San Diego	Ventura
2017				5			
Neofusicoccum australe	2	2		San Luis Obispo	Riverside	San Diego	Ventura
2018				2			
Neofusicoccum mediterraneum	9	9		San Luis Obispo	Riverside	San Diego	Ventura
2017					1		
2018					5		2
Neofusicoccum nonquaesitum	15	15		San Luis Obispo	Riverside	San Diego	Ventura
2017							
2018				11			1
2019				3			
Neofusicoccum australe	30	28	2	San Luis Obispo	Riverside	San Diego	Ventura
2017							
2018			1	6	3	1	2
2019		28		13		2	1
2020			1				2
Neofusicoccum luteum	56	46	10	San Luis Obispo	Riverside	San Diego	Ventura
2017							
2018		21	10	2	1	25	3
2019				18		2	4
2020			1				1
Neofusicoccum parvum	24	24		San Luis Obispo	Riverside	San Diego	Ventura
2018							8
2019				11		4	1

Table 7. Incidence of *Botryosphaeria* on wounded avocado twigs following artificial inoculations with spore suspension of *L. theobromae.*

Inoculum	**Period of inoculation /sampling**	**# Inoculated branches**	***L. theobromae***	
			Incidence (%)	**Mean canker length (cm)**
L. theobromae	Jan20 - May20	10	100	14
	Feb20 – Jun20	10	100	19.5
	Mar20 – Jul20	10	100	44.2
	Apr20 – Aug20	10	100	5.7
	May209 – Sep20	10	100	22.2
	Jun20 – Oct20	10	100	64.7
	Jul20 – Dec20	10	100	83.3
Sterile water	Jan20 - May20	10	0	0
	Feb20 – Jun20	10	0	0
	Mar20 – Jul20	10	0	0
	Apr20 – Aug20	10	0	0
	May19 – Sep20	10	0	0
	Jun20 – Oct20	10	0	0
	Jul20 – Dec20	10	0	0

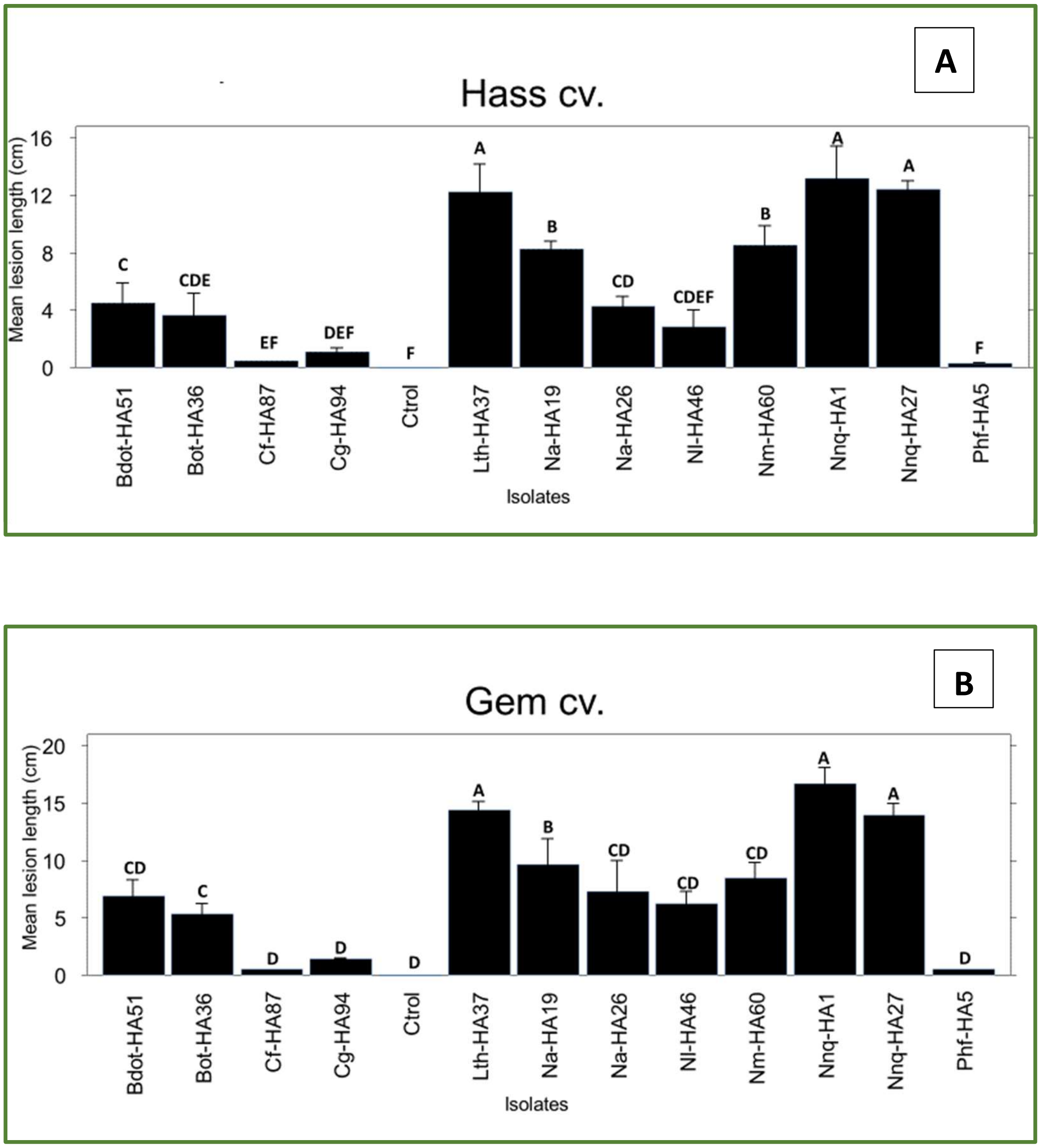

Fig. 1. Canker lesion length (cm) caused by *Botryosphaeria* (Bdot-HA51, Bot-HA36, Lth-HA37, Na-HA19, Na-HA46, Nm-HA60, Nnq-HA1, Nq-HA27), *Colletotrichum* (Cf-HA87, Cg-HA94) and *Phomopsis* (PhF-HA5) species on detached Hass (**A**) and Gem (**B**) avocado twigs 2 weeks after inoculation. Lesion length values followed by the same letter are not significantly different according to LSD test (P = 0.05).

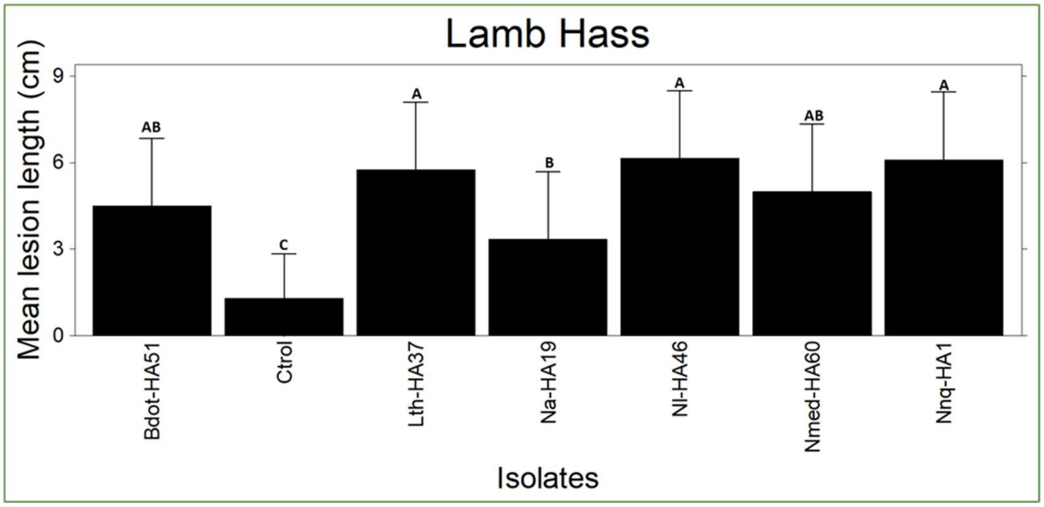

Fig. 2. Mean lesion length (cm) caused by *Botryosphaeria* species on Lamb Hass avocado trees, 2 months after field inoculation. Vertical bars represent the standard error of the mean. Mean lesion length values followed by the same letter are not significantly different according to LSD test (*P* = 0.05).

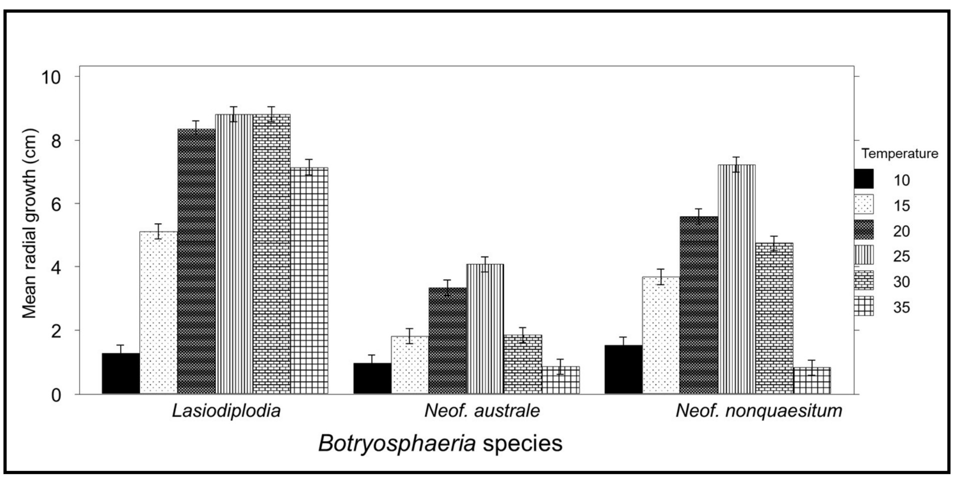

Fig. 3. Effect of temperature on the growth of three *Botryosphaeria* species. Vertical bars represent the standard error of the mean ($P = 0.05$).

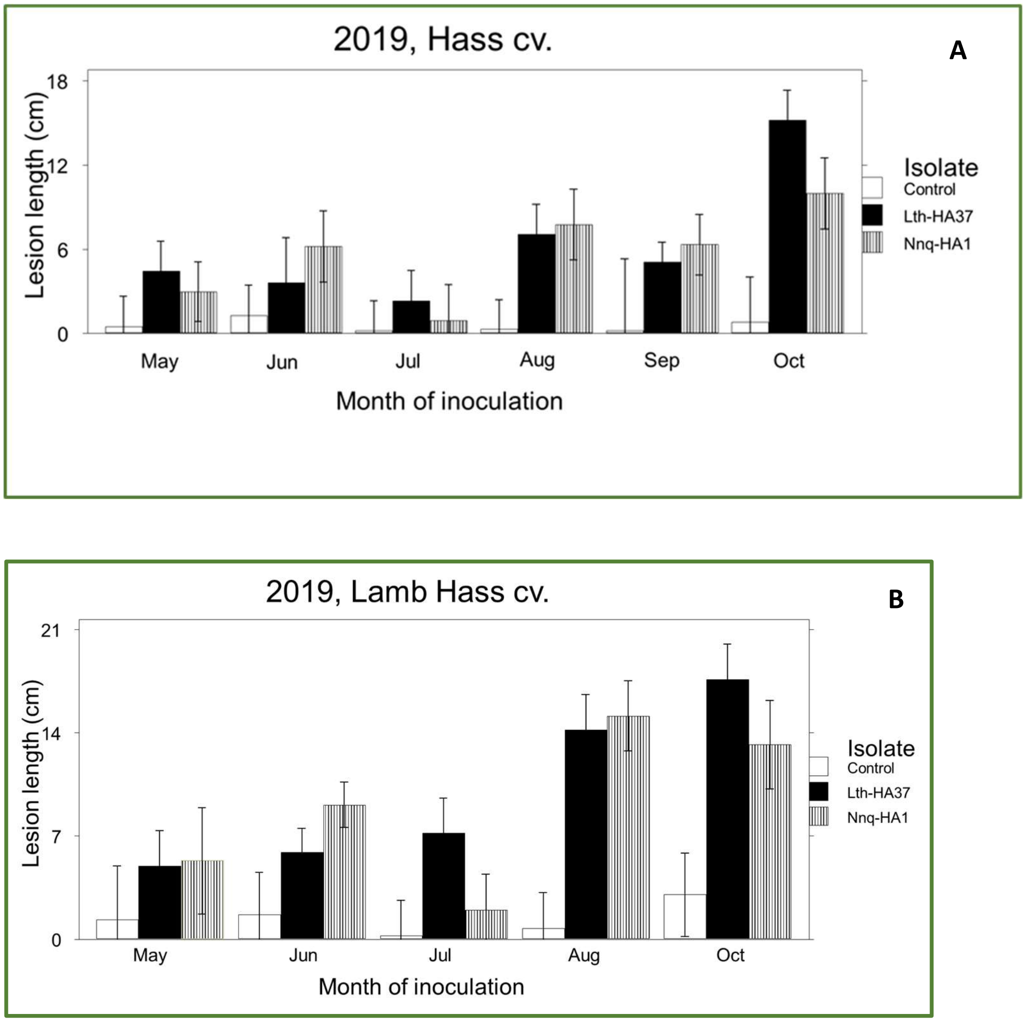

Fig. 4. Canker formation on wounded branches of Hass (**A**) and Lamb Hass (**B**) avocados following inoculation with two *Botryosphaeria* species (Lth-HA37 and Nq-HA1). Inoculations were performed after wounding at the beginning of each month and lengths of internal wood lesion measured two months later. Control are branches inoculated with agar plus free of the pathogens. Vertical bars represent the standard error of the mean (P = 0.05).

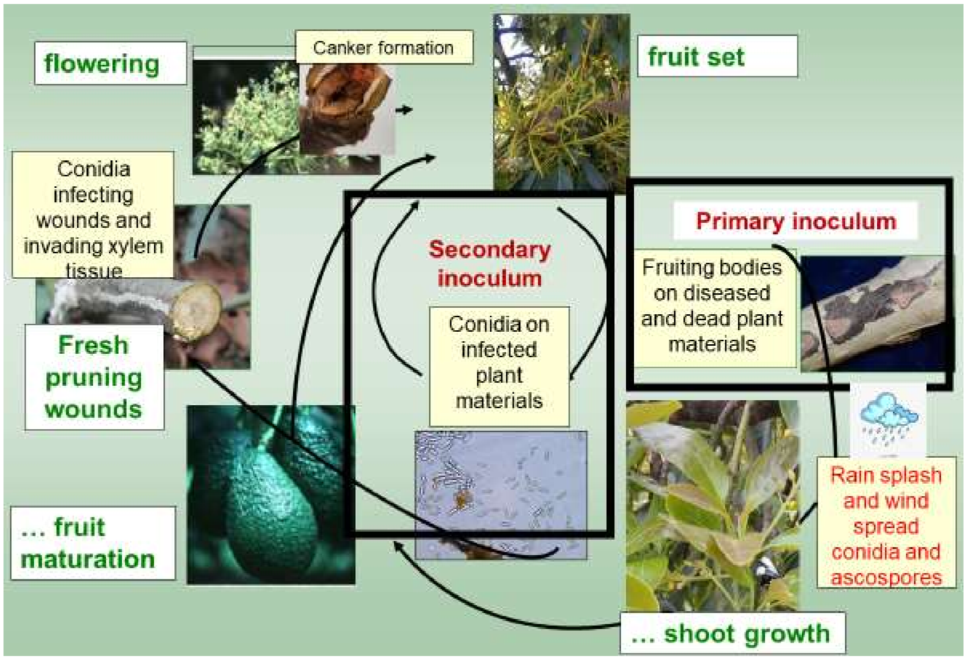

Fig. 5. Proposed Botryosphaeria canker and dieback disease cycle.