Better Growing

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New Findings on Botryosphaeria Branch Canker and Dieback of Avocados in California

Botryosphaeria branch canker and dieback of avocado (*Persea americana* Mill.), commonly referred to as Avocado Branch Canker, 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. 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.

These pathogens can survive as parasites or saprophytes, but many are latent pathogens of woody shrubs and trees that may live undetected in an asymptomatic host until stressful conditions weaken the host and symptoms are expressed. 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. Following hydration during the rainy season, pycnidia release asexual conidia (spores) that are spread by rain splash and wind, disseminating the fungi from tree to tree, and from one part of the tree to another.

Fig. 1. Symptoms of Avocado Branch Canker on Hass: (left) wood discoloration and canker and (right) shoot dieback symptoms.

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 tools 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 **(Figure 1)**. 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.

Once inside the plant, *Botryosphaeria* 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, our plant pathology group at the University of California, Kearney Agricultural Research and Extension 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 also were 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 causes 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 is 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 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.

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

County	City	Number of orchards sampled	Avocado cv [1]	Date of sampling
Ventura	Santa Paula	4	Hass	November 2018
	Pomona	1	Hass	November 2018
	Fillmore	1	Hass	November 2018
	Fillmore	1	Citrus	November 2018
San Diego	Bonsall	1	Lamb Hass	November 2018
	Bonsall	1	Hass	November 2018
	Valley Center	2	Hass	November 2018
	Pauma Valley	1	Lamb Hass	November 2018
	Pauma Valley	1	Hass	November 2018
	Fallbrook	1	Hass	November 2018
Riverside	Riverside	4	Hass	November 2018
San Luis Obispo	Morro Bay	1	Hass	November 2018
	Morro Bay	1		April 2019
	San Luis Obispo	1	Hass	November 2018
Tulare	Exeter	1	Hass	November 2018, April 2019
			Gem	
			Carmen	
			Unreleased avocado cultivars	

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

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 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 (**Figure 2**). Growing fungal isolates subsequently were identified at the genus level based on colony and conidial morphology. The incidence of *Botryosphaeria* and *Colletotrichum* and other recovered fungi were then determined.

Analyses of pathogen incidence data from the surveyed orchards, in both sampling years, showed that *Botryosphaeriaceae* causing ABC were the fungi predominantly associated with

the cankered branches **(Tables 2 and 3)**. These fungi were present in every sampled grove and caused symptoms on all surveyed cultivars, including Hass, GEM, and Lamb Hass **(Tables 2 and 3)**. In addition to woody tissue, these fungi also were detected on other avocado organs, including leaves, flowers, fruit stems (peduncles) and young and mature fruits. Moreover, *Botryosphaeria* also were present in symptomless tissues (flowers, twigs, leaves, fruits), 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.

Fig. 2. Isolation of fungi by plating sections of infected avocado tissues on acidified agar medium.

Table 2. Incidence of Botryosphaeria in cankered avocado branches collected in 2018 from several avocado orchards in California.

				Number (%) of cankered branches yielding [a]				
County	Orchards	Cultivar	Branch cankers [b]	*Botryosphaeria*	*Colletotrichum*	*Phomopsis*	*Alternaria*	*Fusarium*
Riverside	RC1	Hass	192	49	17	0	34	12
	RC2		101	42	1	11	30	22
	RC3		50	26	4	0	56	12
Ventura	PTR	Hass	263	62	18	0	7	9
	G3		104	84	9	0	0	5
	LIM High density		162	21	30	9	4	27
	LIM-O		32	53	44	0	0	0
	DA1		180	50	26	0	12	1
San Diego	West-L	Hass	117	80	11	0	1	8
	ZRT		42	57	17	0	0	10
	Mesa-C		63	29	21	3	14	16
	Starbeam		51	20	6	2	29	39
	NIG		32	6	9	0	16	69
	West-L	Lamb	94	67	27	0	10	15
	Starbeam	Hass	53	76	6	0	8	15
San Luis Obispo	GR1	Hass	38	90	14	0	0	0
	GR2		223	82	9	0	1	0
Tulare	Lindcove Station	Hass	70	1	0	0	36	4
		Gem	10	10	0	0	70	0
		Carmen	30	3	7	0	13	0
			Total= 1907	**45**	**14**	**1**	**17**	**13**

[a] *Branch samples per orchard yielding indicated fungi. Numbers in bold are averages per orchard.*

[b] *Number of cankered branches collected in each orchard.*

Table 3. 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

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 4)**. 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 3 and 5)**. 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: **(Figure 3)** and *Colletotrichum* (acervulli and perithecia) were observed on both living and dead tissues (branches and leaves) from the orchards. This further

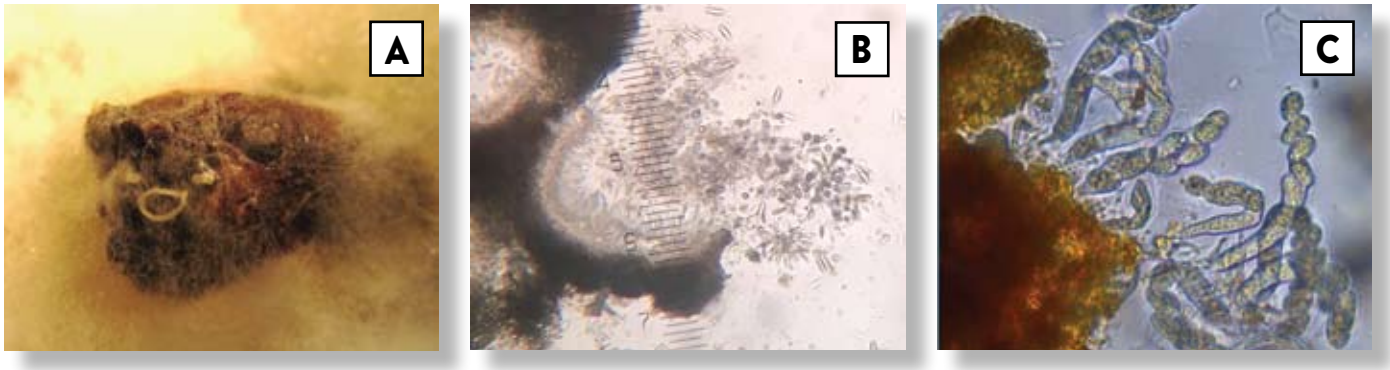


Fig. 3. Oozing pycnidia of Botryosphaeria on dead avocado branch (A); Pycnidia releasing spores in rainy conditions (B); and pseudothecia in avocado tissues with windborne ascospores (C).

Table 4. Incidence (%) of Botryosphaeria 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
	PTR		20	0	0	0	80	0
Ventura	G3	Hass	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
	West-L		42	41	36	0	14	7
San Diego	ZRT	Hass	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 5. Incidence (%) of Botryosphaeria sp. in symptomatic avocado tissues collected in Spring of 2019 from four avocado orchards located in San Luis Obispo County.

		Infected tissue sample		Pathogen incidence (%) [b]				
Orchard	Cultivar	Type	Number [a]	*Botryosphaeria*	*Colletotrichum*	*Phomopsis*	*Alternaria*	*Fusarium*
GR1	Hass	Leaves	101	19	73	1	11	6
GR3			50	46	12	4	22	0
GR4			80	6	76	9	53	1
GR2			160	22	26	4	28	0
			Total = 391	**23**	**47**	**5**	**29**	**2**
GR1		Twigs	161	67	24	10	6	3
GR3			100	85	5	1	2	6
GR4			50	24	24	14	16	4
GR2			163	82	7	14	5	9
			Total = 474	**65**	**15**	**10**	**7**	**6**

[a] *Number of infected leaves collected in each orchard; numbers in bold are averages per orchard.*

[b] *Tissue samples per orchard yielding indicated fungi.*

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*, also were isolated from the samples, but at lower proportions (**Tables 2, 3, and 5**).

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

The use of molecular methods allowed the classification of 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. Once the fungal species were identified we wanted to know which ones were more aggressive on avocados. This was first done by assessing the isolates' pathogenicity on healthy, detached green shoots (1-year-old) of Hass and GEM varieties of avocado. Excised 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. Shoots were in-

cubated at room temperature (~75.2˚F) and under humid conditions for two weeks. Resulting canker lesion lengths were measured and isolations were made from these shoots to confirm pathogenicity.

Our DNA analyses of the recovered fungi confirmed that common species (about 11 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. 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) **(Figure 4)**. Furthermore, inoculations of wounded detached shoots of various unreleased avocado cultivars, grown at the Lindcove REC, showed that they are susceptible to *Botryosphaeria* infection. All of the six species of the Botryosphaeriaceae were also found to be more aggressive than the *Colletotrichum* and *Phomopsis* species **(Figure 4)**. 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. 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. Results from this 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 5)**. 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

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 also were assessed. Pathogen inoculum availability on green, symptomless tissue (latent infections) also was monitored after surface-disinfestation. Monthly, artificial inoculations were performed with mycelial plugs of two *Botryosphaeria*

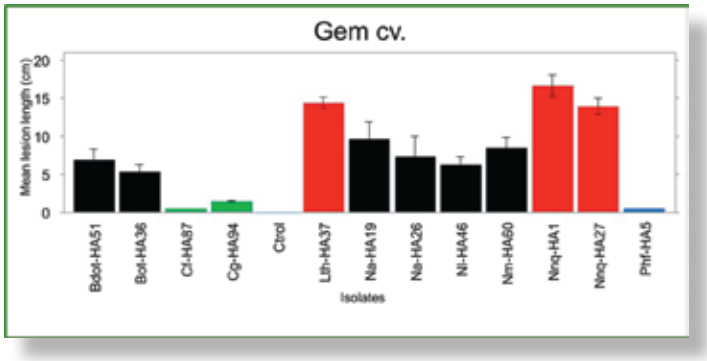


Fig. 4. Canker lesion length (cm) caused by Botryosphaeria (black and red bars = most virulent), Colletotrichum (green bars) and Phomopsis (blue bar) species on detached GEM avocado twigs 2 weeks after inoculation. Vertical bars represent the standard error of the mean (P = 0.05).

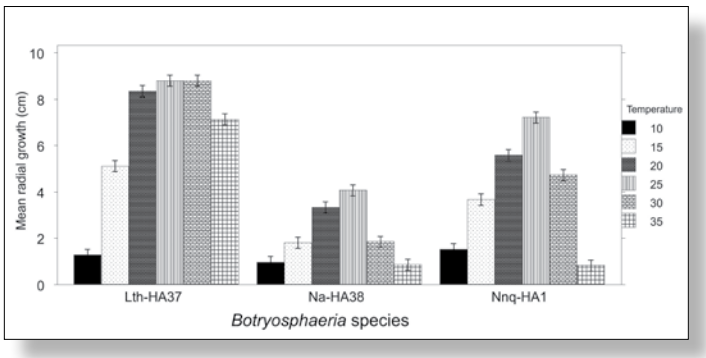


Fig. 5. Effect of temperature on the growth of three Botryosphaeria species. Vertical bars represent the standard error of the mean (P = 0.05).

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 two and four months after inoculations, respectively.

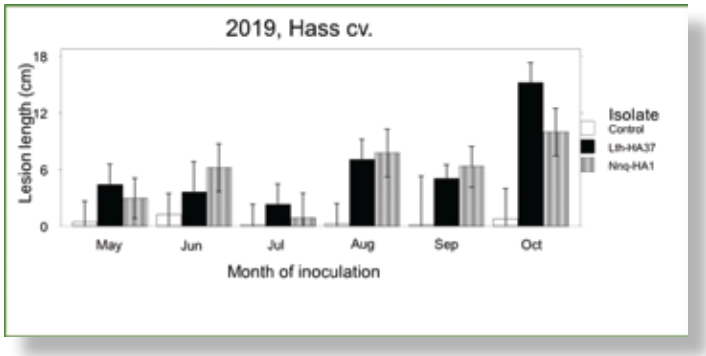


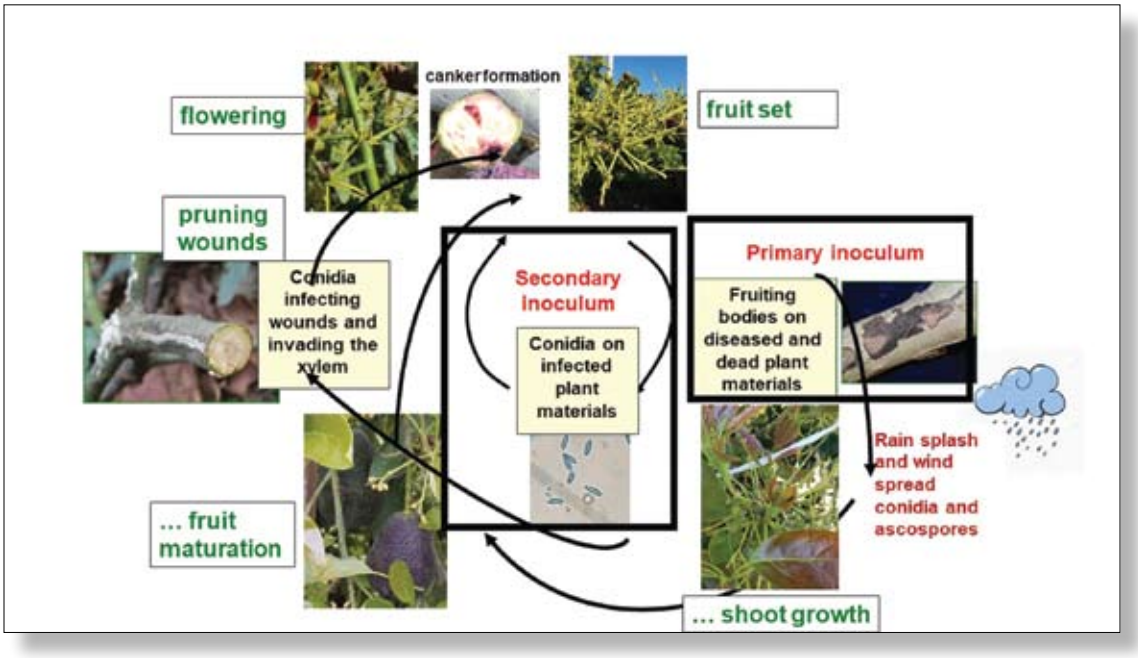
Fig. 6. Canker formation on wounded branches of Hass avocado following inoculation with two Botryosphaeria species (Lth-HA37 and Nqq-HA1). Control are branches inoculated with agar plus free of the pathogens. Vertical bars represent the standard error of the mean (P = 0.05).

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 **(Figure 6)**. Inoculations with spores resulted in the development of canker lesions on wounded branches. 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 periods of high humidity underscores 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.

Effects of water stress on infection

The influence of water and heat stress factors on disease initiation and development by Botryosphaeriaceae on avocado also were 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 symptom-

Fig. 7. Proposed Botryosphaeria canker and dieback disease cycle.



atic twigs were plated on acidified agar medium followed by isolations of the associated fungi. Results 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.

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 two months after inoculation with *L. theobromae*. Stressed avocado trees had larger canker lesion sizes compared to the control plants (data not shown).

Conclusions and recommendations

This study shows that:

Botryosphaeria are prevalent in avocado-producing regions of California and are the primary fungal pathogens associated with ABC; it also led to a better understanding of the pathogen life and disease cycles as illustrated in (**Figure 7**).

Inoculum is 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.

Adherence to best management practices recommended for the management of canker and dieback pathogens also should be followed for managing ABC in avocado groves. These practices include:

a) avoid pruning during or immediately after rain, dew or heavy fog;
b) pruning 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 pruning dense canopies to increase air flow and reduce humidity;
e) reducing tree stress and maintaining trees in good condition through appropriate irrigation and fertilization practices.

The utilities of various fungicides in protecting pruning wounds against ABC infection and of kaolin-based products in providing a physical barrier against sunburn injuries are under investigation. These chemical and physical approaches may become part of an integrated management strategy against this disease. Additional work was performed during the second year of the project to investigate the prevalence of ABC pathogens in newly established orchards and the significance of nursery inoculum in ABC epidemiology in young trees to gain further insights into controlling these pathogens.

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